Volume No. 8 Issue No. 1

21, July 2024

ISSN 2789-2093.

TUJMS

Tobruk University Journal Of Medical Sciences

Editorial Board

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Content

Tobruk University Journal of Medical Sciences (TUJMS): ISSN No 2789-2093 Volume (8): Issue 1 July 2024
Original Article

Optic Disc and Macular Vessel Density Measured by Optical Coherence Tomography-Angiography in Diabetic Patients

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https://medicine.tu.edu.ly/journal/4514/

Received: 01/05/2024 Accepted: 22/05/2024

ABSTRACT:

Introduction: Diabetes mellitus is a global epidemic, affecting millions of individuals and posing substantial challenges to public health worldwide. Beyond its systemic impact, diabetes gives rise to a myriad of complications, with diabetic retinopathy (DR) emerging as a primary cause of visual impairment and blindness, particularly among the working-age population. DR is a progressive microvascular complication characterized by alterations in the retinal vasculature that can lead to severe vision loss if left untreated. The optic disc and macula, critical components of the retina, are particularly susceptible to these vascular changes, making their comprehensive evaluation imperative for understanding the trajectory of diabetic retinopathy. Optical Coherence Tomography-Angiography (OCT-A) has made it possible to assess and measure early microvascular damage in diabetes, such as capillary nonperfusion and ischemia. While various factors alter the vascular tissue's shape, little is known about how the ocular, systemic, and demographic aspects of diabetes patients affect OCT-A metrics. The study aims to assess the capabilities of Optical Coherence Tomography Angiography (OCTA) to comprehensively investigate optic disc and macular vascular changes in diabetic patients.

Materials and methods: Eighteen, 11 Type 1 and 7 Type 2 diabetic patients with or without prior ocular pathology and eight matching healthy individuals were recruited from the clinics at Tobruk Medical Centre. Clinical refractometry, fundus examinations, and imaging by OCT were performed.

Results: The foveal thickness was not statistically significant among the studied groups (p =.275), but the central foveal thickness showed a statistical difference among the studied groups (*p =.043*). The intergroup pairwise comparison revealed that the proliferative diabetic retinopathy (PDR) group had statistically significantly lower central foveal thickness than the severe DR group (*p =.045).*

Conclusion: Different OCT measurements, including focal thickening, may be useful for the early detection of macular thickening and may be indicators for a closer follow-up for diabetic patients.

Key Words: Optical Coherence Tomography, Optic Disc, Macular Vascular-Diabetic Retinopathy

INTRODUCTION

Worldwide, nearly half a billion individuals (9.3% of adults aged 20–79 years) are currently living with Diabetes Mellitus (DM). [1] Among them, approximately one-third are believed to have Diabetic Retinopathy (DR), with a further one-third of those progressing to vision-threatening complications such as Proliferative DR (PDR) or Diabetic Macular Edema (DME). [2]

Given the substantial economic impact of this condition, effective management relies on early detection and accurate staging, which in turn depends on utilizing the most suitable imaging technology. [2]

Sun et al.,[3] highlight the necessity for an updated DR grading system that integrates systemic health status alongside modern multimodal imaging techniques. This underscores the importance of developing a quantitative DR staging system to enhance diagnostic accuracy and guide treatment decisions effectively. Advanced stages of DME and DR develop as a result of increased vascular permeability and pathologic neovascularization triggered by microvascular injury. [4]

Optical Coherence Tomography-Angiography (OCT-A) has emerged as a non-invasive alternative to Fluorescein Angiography (FA); with no intravenous dye injection, optical coherence tomography-angiography (OCT-A) has been developed to provide depth-resolved visualization of the retinal microvasculature. OCT-A is based on mapping erythrocyte movement over time by comparing sequential OCT B-scans (motion contrast) at a given cross-section[5,6]

OCT-A can identify microvascular abnormalities in diabetic eyes, such as microaneurysms, capillary dropout, neovascularization, and enlargement of the Foveal Avascular Zone (FAZ), in good agreement with fluorescein angiography. [7-11] Additionally, FAZ size and vessel density have been assessed in some of these investigations and linked with the severity of diabetic macular ischemia, indicating the enormous potential of microvascular alterations, and specifically quantitative metrics derived from OCT-A, as biomarkers of diabetic macular and vascular changes.

As the entry point for optic nerve fibers, the optic disc and the macula, responsible for high-acuity vision, are pivotal regions in the retina intimately connected to visual function. Consequently, alterations in the vascular supply to these regions can have profound implications for diabetic patients. Previous research has emphasized the potential of OCTA in revealing early microvascular changes associated with diabetes. [12] However, a comprehensive exploration specifically focusing on the optic disc and macula is essential to bridge existing knowledge gaps and inform clinical practice.

This study aligns with global efforts to enhance diabetic eye care, as recommended by the American Diabetes Association and the International Council of Ophthalmology. [13,14] By positioning itself at the intersection of ophthalmology and endocrinology, this research endeavors to contribute to academic understanding and the practical improvement of patient outcomes through targeted interventions. The study aims to utilize the capabilities of Optical Coherence Tomography Angiography (OCT-A) to comprehensively investigate optic disc and macular vascular changes in diabetic patients.

The primary objective was to assess vascular alterations by employing OCT-A, and meticulously evaluate and quantify vascular alterations in both the optic disc and macula of diabetic patients. This includes a comprehensive analysis of vessel density, perfusion density, and characteristics of the foveal avascular zone, while the secondary objective was to correlate

changes with the severity and duration of diabetes mellitus.

MATERIALS AND METHODS

Study Design:
A Cross-sectional diagnostic study was conducted at the Ophthalmology Department, Tobruk Medical Centre, Libya.

Participants:
Eighteen, 11 Type 1, and 7 Type 2 diabetic patients, with or without prior ocular pathology, were recruited from the Clinics at Tobruk Medical Centre. Informed consent was obtained from each participant and eight matching healthy individuals.

Selection Criteria for Patients:
Inclusion criteria:

- Age range between 18 to 75 years
- Stratification is based on the severity of diabetic retinopathy (mild, moderate, severe).

Exclusion criteria:
Patients presenting with concurrent ocular conditions such as uveitis or glaucoma detected during the ocular examination were excluded from the analysis. Additionally, individuals who cannot undergo retinal imaging and ocular examinations (including OCT, OCTA, fundus retinographies, biometry, etc.) or provide written informed consent were excluded.

Imaging Technique:
Huvitz Optical Coherence Tomography HOCT-1 was utilized to capture detailed optic disc and macular vasculature images.

Clinical Examination:
Comprehensive clinical examinations included

- Best-Corrected Visual Acuity Assessment
- Measurement of Visual Acuity using standardized charts.
- Intraocular Pressure Measurement (utilizing a tonometer).
- Anterior Segment Evaluation
-

- A slit-lamp biomicroscope is used for a detailed examination of the anterior segment.

Refractometer Examination:
An auto-refractometer was utilized to account for potential refractive errors impacting diabetic retinopathy progression.

Fundus Examination:
A dilated fundus examination by a trained ophthalmologist will be conducted to assess the optic disc, macula, and retinal vasculature. This examination will specifically look for signs of diabetic retinopathy, such as microaneurysms, hemorrhages, exudates, and neovascularization.

Imaging Studies:
OCT and OCT-Angiography were conducted to detect foveal thickness (mean thickness in the central 1000-μm diameter area), central foveal thickness (mean thickness at the point of intersection of 6 radial scans), microvascular changes, capillary dropout, and neovascularization.

RESULTS

Age and sex were statistically matched among studied groups (p=.506, p=.581, respectively). Out of the diabetic population (n=18); 11 (61.11%) were with Type I DM, and 7 (38.89%) were with Type II. In Type I DM patients, the disease duration ranged from 2.00 to 30.00 years with a median of 15.00 years; in Type II DM patients, it ranged from 2.00 to 20.00 years with a median of 12.00 years. In the Type I DM patients, the HbA_{1c} ranged from 4.50 to 10.00% with a median of 8.50%, while in the Type II DM Group, it ranged from 5.90 to 10.00% with a median of 8.00% (Table 1).

The VA best-corrected logMAR showed a statistically significant difference among the studied groups (p<.001). The intergroup pairwise comparison revealed that the VA best-corrected logMAR in the DM with no DR Group was statistically significantly lower than the Control and PDR groups (p=.013 and p=.020, respectively). In addition, the Moderate NPDR Group was statistically significantly lower than the Control Group and the Severe NPDR Group (p=.012 and p=.021, respectively). Although the IOP had a statistically significant difference among the studied groups (p=.027), the inter-group pairwise comparison revealed no statistically significant difference between any pair. There was no statistically significant difference in foveal thickness (µm) among the studied groups (p=.275). Central foveal thickness (µm) differed statistically significantly among the studied groups (p=.043). The inter-group pairwise comparison revealed that central foveal thickness in the PDR Group was statistically significantly lower compared with the DM in the Severe DR Group (p=.045) (Table 2).

Superficial Retinal Vascular Plexus (%) showed no statistically significant difference among the studied groups (p=.282). There was a statistically significant difference in Deep Retinal Vascular Plexus (%) among the studied groups (p=.003). The inter-group pairwise comparison revealed that it was statistically significantly lower in the DM no DR Group than in the Control Group and the Moderate NPDR Group (p=.005 and p=.011, respectively). There was no statistically significant difference in Choriocapillaris Vascular Density among the studied groups (p=.052). Also, Vessel Density at the Optic disc (Superficial) had no statistically significant difference among the studied groups (p=.971). Vessel Density at the Optic disc (Deep) had no statistically significant difference among the studied groups (p=.988) (Table 3).

An example of Macula OCT-A of a 60-year-old female with Type I Diabetes Mellitus with proliferative diabetic retinopathy is illustrated in Figure (1).

Table (1): Demographic and diabetes-related data of the studied groups

Demographic data	Total	Group						
		Control	DM No DR	Mild NPDR	Moderate NPDR	Severe NPDR	PDR	Test of significance *p*-value
Age (years)								
- n	26	8	4	5	4	2	3	
- Min. – Max.	38.00-75.00	45.00-75.00	48.00-70.00	58.00-75.00	46.00-72.00	54.00-60.00	38.00-64.00	$H_{(df=5)}$=4.310
- Median	59.00	53.50	59.00	62.00	61.50	57.00	60.00	*p*=.506 NS
- 95% CI of the median	54.00-62.00	47.00-60.00	48.00-70.00	61.00-75.00	46.00-72.00	54.00-60.00	38.00-64.00	
- 25th Percentile –75th Percentile	52.00-62.00	47.50-57.50	52.00-66.00	61.00-62.00	49.50-71.00	54.00-60.00	38.00-64.00	
Sex n (%)								
- **Male**	7 (26.92%)	3 (37.50%)	0 (0.00%)	1 (20.00%)	2 (50.00%)	0 (0.00%)	1 (33.33%)	$\chi^2_{(df=5)}$=3.933
- **Female**	19 (73.08)	5 (62.50%)	4 (100.00%)	2 (80.00%)	2 (50.00%)	2 (100.00%)	2 (66.67%)	$p_{(MC)}$ =.581
	Type I DM				**Type 2 DM**			
Type of diabetes (n=18)	11 (61.11%)				7 (38.89%)			
Duration of DM (years)								
- Min. – Max.	2.00-30.00				2.00-20.00			
- Median	15.00				12.00			
- 95% CI of the median	8.00-23.00				10.00-20.00			
- 25th Percentile –75th Percentile	8.00-23.00				3.00-14.00			
HBA_{1c} (%)								
- Min. – Max.	4.50-10.00				5.90-10.00			
- Median	8.50				8.00			
- 95% CI of the median	7.40-10.00				6.50-10.0			
- 25th Percentile –75th Percentile	7.40-10.00				6.50-1.00			

DM: Diabetes Mellitus **DR:** Diabetic Retinopathy **NPDR:** Non-Proliferative
N: Number of patients **Min-Max:** Minimum – Maximum **CI:** Confidence interval
H: Kruskal-Wallis H χ^2=Pearson Chi-Square **MC:** Monte Carlo correction
DF: degree of freedom. ***:** Statistically significant ($p<.05$) **NS:** Statistically not significant ($p\geq.05$)

Table (2): Clinical and Macular OCT Findings in the studied groups

	Group						Test of significance *p*-value
	Control (n=16) a	**DM No DR (n=8) b**	**Mild NPDR (n=10) c**	**Moderate NPDR (n=8) d**	**Severe NPDR (n=4) e**	**PDR (n=4) f**	
VA best-corrected logMAR							
- Min. – Max.	0.700-0.800	0.100-0.400	0.000-1.000	-0.300-0.600	0.800-1.000	0.200-1.000	
- Median	0.700a^{cef}	0.200b^{cdf}	0.350^{abcdef}	0.250^{bcdf}	0.900^{acfe}	0.900^{abcdef}	$H_{(df=5)}$=28.855
- 95% CI of the median	0.00-0.00	0.200-0.400	0.200-0.500	0.200-0.400	0.800-1.000	0.900-1.000	*p*<.001*
- 25th Percentile –75th Percentile	0.700-0.800	0.200-0.400	0.200-0.500	0.200-0.350	0.800-10.000	0.550-0.950	
IOP (mmHg)							
- Min. – Max.	10.00-14.00	11.00-14.00	11.00-14.00	11.00-15.00	12.00-15.00	14.00-15.00	
- Median	12.00a^{bcdef}	11.00^{abcdef}	13.00^{abcdef}	12.00^{abcdef}	15.00^{abcdef}	14.50^{abcdef}	$H_{(df=5)}$=12.656
- 95% CI of the median	12.00-13.00		13.00-14.00	11.00-15.0	15.00-15.00	14.00-15.00	*p*=.027*
- 25th Percentile –75th Percentile	11.50-13.00	11.00-14.00	12.00-14.00	11.00-14.00	12.00-15.00	14.00-15.00	
Foveal Thickness (µm)							
- Min. – Max.	152.990-501.050	186.090-271.380	175.400-647.900	146.370-310.140	224.100-640.340	179.370-237.900	
- Median	207.455	199.320	220.480	217.525	295.580	183.250	$H_{(df=5)}$=6.331
- 95% CI of the median	204.150-220.980	190.630-271.380	187.410-250.670	182.300-267.690	224.100-640.340	179.370-237.900	*p*=.275 NS
- 25th Percentile –75th Percentile	202.870-234.500	190.630-237.620	187.410-250.670	191.520-248.825	224.100-640.340	180.270-211.615	
Central Foveal Thickness (µm)							
- Min. – Max.	174.050-502.330	226.750-271.360	201.120-588.850	212.190-318.310	270.970-619.390	224.260-240.660	
- Median	250.885a^{bcdef}	248.930^{abcdef}	232.920^{abcdef}	266.260^{abcdef}	320.540^{abde}	228.365^{abcdf}	$H_{(df=5)}$=11.461
- 95% CI of the median	240.810-267.790	228.200-271.360	213.340-264.910	239.270-286.120	270.970-619.390	224.260-240.660	*p*=.043*
- 25th Percentile –75th Percentile	240.445-274.360	228.200-264.860	213.340-264.910	247.030-282.900	270.970-619.390	225.055-235.770	
Lens							
- Normal	12 (75.00%)	2 (25.00%)	0 (0.00%)	0 (0.00%)	1 (25.00%)	0 (0.00%)	$\chi^2_{(df=10)}$=38.125
- Pseudophakia	4 (25.00%)	6 (75.00%)	5 (50.00%)	4 (50.00%)	0 (0.00%)	1 (25.00%)	$p_{(MC)}$ <.001*
- Cataract	0 (0.00%)	0 (0.00%)	5 (50.00%)	4 (50.00%)	3 (75.00%)	3 (75.00%)	

n: Number of eyes **Min-Max:** Minimum – Maximum **CI:** Confidence interval **H:** Kruskal-Wallis H

χ^2=Pearson Chi-Square **df:** degree of freedom *: Statistically significant (*p*<.05) **NS:** Statistically not significant (*p*≥.05

Post-hoc pairwise comparison among groups: Each group assigned a letter by default: (a) to (f). Different superscript letters indicate statistically significant differences at *p*<.05, adjusted according to the Bonferroni method.

Table (3): Macular OCT-Angio Findings in the studied groups

	Group						Test of significance *p*-value
	Control (n=16)	DM No DR (n=8)	Mild NPDR (n=10)	Moderate NPDR (n=8)	Severe NPDR (n=4)	PDR (n=4)	
Superficial Retinal Vascular Plexus (%)							
- Min. – Max.	27.851-44.747	15.860-38.249	27.371-43.347	27.120-35.646	28.159-34.210	27.820-32.363	
- Median	28.221	28.199	28.212	28.215	28.208	28.213	$H_{(df=5)}$=6.256
- 95% CI of the median	28.201-28.484	28.191-28.215	28.196-28.361	28.201-28.691	28.198-28.586	28.193-28.463	*p*=.282 NS
- 25th Percentile –75th Percentile	28.192-29.295	23.247-28.397	28.194-28.753	28.196-29.906	28.199-28.454	28.192-28.477	
Deep Retinal Vascular Plexus (%)							
- Min. – Max.	25.760-44.437	15.714-38.152	27.077-43.364	28.019-35.817	27.529-34.201	28.155-32.367	
- Median	28.258a[cdef]	28.196b[cdef]	28.208[abcdef]	28.364[abcdef]	28.200[abcdef]	28.368[abcdef]	$H_{(df=5)}$=17.753
- 95% CI of the median	28.213-28.513	28.186-28.208	28.191-28.304	28.202-28.935	28.180-32.149	28.204-28.629	*p*=.003*
- 25th Percentile –75th Percentile	28.200-29.273	17.582-28.216	28.184-28.566	28.200-30.741	28.183-30.234	28.200-29.020	
Choriocapillaris Vascular Density (%)							
- Min. – Max.	26.012-44.034	15.857-38.233	26.119-38.233	20.806-35.661	12.090-34.204	27.860-29.367	
- Median	28.211	28.196	28.202	28.294	28.750	28.207	$H_{(df=5)}$=10.991
- 95% CI of the median	28.199-28.297	28.182-28.209	28.191-28.224	28.198-28.689	28.185-31.356	28.190-28.245	*p*=.052 NS
- 25th Percentile –75th Percentile	28.193-29.304	17.114-28.222	28.185-28.538	28.195-30.394	28.191-31.356	28.190-28.253	
VD at Optic disc (Superficial)							
- Min. – Max.	20.588-53.155	13.240-47.941	27.664-34.305	0.000-44.260	7.365-44.030	28.190-44.971	
- Median	28.236	28.214	28.214	28.456	28.203	28.289	$H_{(df=5)}$=0.890
- 95% CI of the median	28.199-28.540	28.195-28.406	28.200-29.244	28.197-29.368	28.189-33.540	28.204-28.910	*p*=.971 N
- 25th Percentile –75th Percentile	28.196-30.188	28.192-29.129	28.196-30.526	28.194-29.651	28.194-31.784	28.202-29.303	
VD at Optic disc (Deep)							
- Min. – Max.	25.902-47.084	25.667-41.022	25.667-34.199	0.000-41.470	14.029-44.060	28.185-41.450	$H_{(df=5)}$=0.590
- Median	28.324	28.375	28.246	28.291	28.199	28.236	*p*=.988 NS
- 95% CI of the median	28.203-28.587	28.202-28.588	28.201-28.781	28.198-28.614	28.194-32.654	28.204-28.401	
- 25th Percentile –75th Percentile	28.196-29.407	28.197-30.761	28.197-31.026	28.196-29.579	28.194-30.797	28.202-29.045	

n: Number of eyes **Min-Max:** Minimum – Maximum **CI:** Confidence interval **H:** Kruskal-Wallis H

χ^2=Pearson Chi-Square **df:** degree of freedom ***:** Statistically significant ($p<.05$) **NS:** Statistically not significant ($p\geq.05$)

Post-hoc pairwise comparison among groups: Each group assigned a letter by default: (a) to (f). Different superscript letters indicate statistically significant differences at $p < .05$ adjusted according to the Bonferroni method.

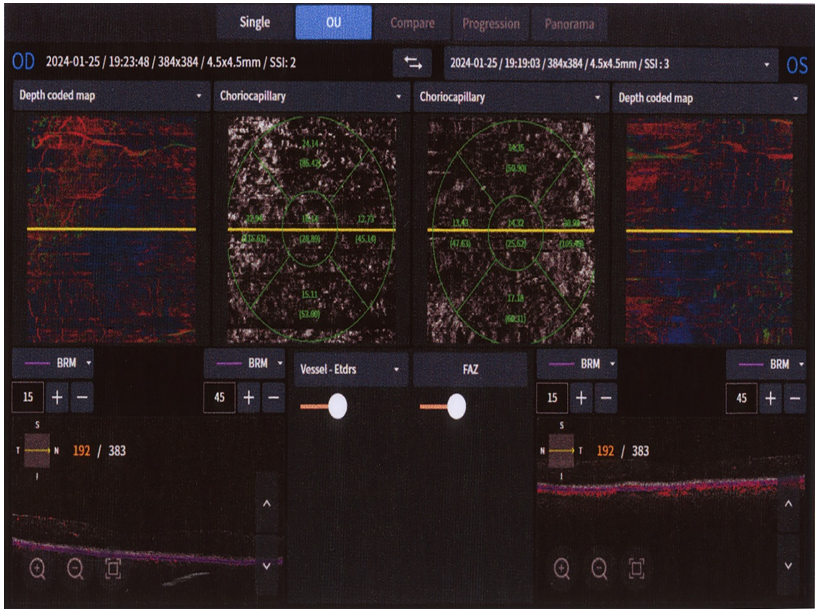

Figure (1-A)

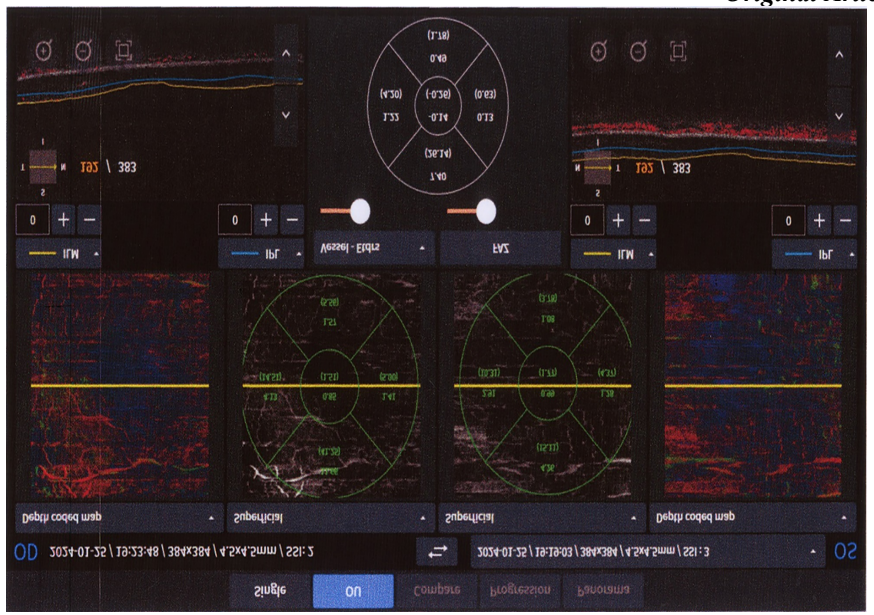

Figure (1-B)

Figure (1): Macula OCT-A of a 60-year-old with Type I Diabetes Mellitus for 23 years; her HbA_{1c} is 10. She had an eye for proliferative Diabetic retinopathy, showing decreased vessel density of the retinal superficial capillary plexus of macula and choriocapillaris.

DISCUSSION

Our study aims to assess the capabilities of Optical Coherence Tomography Angiography (OCTA) to comprehensively investigate optic disc and macular vascular changes in diabetic patients.

In the present study, Although the IOP was statistically significantly different among the studied groups (p=.027), the inter-group pairwise comparison revealed no statistically significant difference between any pair.

Khorasani et al. (2020),[15] reported that an acute increase in IOP of approximately 13 mmHg impairs optic disc perfusion, as evidenced by decreased inside disc vessel density in healthy and diabetic eyes. However, there was no significant change in peripapillary vessel density following the IOP elevation. The microvascular perfusion within the optic disc primarily stems from the posterior ciliary artery, while the central retinal artery supplies the nerve fiber layer. This suggests that microvascular perfusion originating from the choriocapillaris may be more vulnerable to damage after acute IOP elevation than retinal microvasculature.

We found that the VA best-corrected logMAR statistically differed significantly among the studied groups (p<.001). In the Boned-Murillo et al. (2021) study,[16] the mean VA best-corrected logMAR in the type 2 diabetes mellitus group with moderate diabetic retinopathy (DR) and no diabetic macular edema (DME) was satisfactory. However, the control group exhibited slightly superior values. However, statistical analysis revealed no significant difference between the two groups (p=.06), indicating well-maintained visual function in visual acuity.

In the present study, there was no statistically significant difference among the studied groups in the foveal thickness (p=.275). In contrast to Goebel et al. (2002),[17], who performed a controlled study to measure the thickness of the macular retina in diabetic retinopathy utilizing OCT. They demonstrated that the mean retinal thickness in the fovea of diabetic patients was significantly more significant compared to the control group; within the subgroup of diabetic eyes with Clinically Significant Macular Edema (CSME), retinal thickening was more pronounced. However, in the subgroup of diabetic eyes without CSME, no significant differences were observed in foveal or average retinal thickness compared to control eyes.

Asefzadeh et al. (2008),[18] included a larger sample size and more extensively sampled retinal thickness points. They did not find any significant differences in retinal thickness across various sectors of the macula among the Control, No DR, and Mild DR groups.

Boned-Murillo et al. (2021),[16] detected no discrepancies between the type 2 diabetes mellitus group with moderate diabetic retinopathy (DR) and no Diabetic Macular Edema (DME) across any of the quadrants of the Early Treatment Diabetic Retinopathy Study (ETDRS) grid. Notably, the fovea (ETDRS C) exhibited the lowest thickness in both groups, with the thinnest sector being the outer inferior quadrant. Consistent with previous reports, inner macular regions generally exhibited greater thickness than outer areas, with nasal thickness greater than temporal and superior thickness greater than inferior. [19]

The current study revealed a statistically significant difference in the central foveal thickness among the groups (p=.043). The inter-group pairwise comparison showed that the PDR Group was statistically significantly lower than the DM with Severe DR Group (p=.045).

Network DRCR (2007)[20] reported that the Central Macular Thickness (CMT) in eyes with

severe NPDR to PDR was markedly higher than that in eyes with mild-to-moderate NPDR. These findings suggest that in eyes with severe NPDR and PDR, retinal ischemia extends to the macular area, releasing endogenous vascular permeability factors that disrupt the blood-retina barrier, resulting in substantial leakage from damaged capillaries. Alternatively,[21] microvascular alterations associated with the progression of DME may manifest earlier within the DVP compared to the SVP. Firstly, based on the severity grading of DME in the present investigation[22]

It's important to note that the analysis of retinal thickness in patients with DR compared to healthy individuals often yields varied results due to disease progression and the absence of a consistent pattern. Changes in thickness can occur due to neural tissue loss, vascular permeability, and inflammation, which may counteract the effects of neurodegeneration on macular thickness, as suggested by previous studies. However, increased macular thickness alone may not necessarily indicate the absence of an associated neurodegenerative process. Therefore, relying solely on macular thickness may not be sufficient for detecting early changes in the retina of patients with type 2 DM, as it lacks the required sensitivity. [23-27]

In the current study, Superficial Retinal Vascular Plexus showed no statistically significant difference among the studied groups (p=.282). There was a statistically significant difference in Deep Retinal Vascular Plexus (%) among the studied groups (p=.003).

The literature presents conflicting evidence regarding whether Superficial Capillary Plexus (SCP) or Deep Capillary Plexus (DCP) correlates with DR progression. Ong et al.[28] demonstrated that Vessel Density (VD) changes in DCP were not evident between consecutive stages but were noticeable between study groups separated by two or more stages. Conversely, a decline in SCP VD occurred during early (mild NPDR vs. no DR) and late NPDR (severe NPDR vs. moderate NPDR) progression. Research indicates that the DCP is particularly susceptible to ischemia due to its location in the watershed zone of oxygen supply. [29]

Veiby et al. (2020)[30] reported that the decline in VD within the SCP and DCP represents an early manifestation in DR, detectable before the onset of observable retinopathy. Notably, traditional OCT measurements such as Total Retinal Volume (TRV) and Central Macular Thickness (CMT) showed no association with NPDR progression, highlighting the superiority of OCTA in detecting changes associated with NPDR advancement in the absence of macular edema. [30]

Veiby et al. (2020) [30] findings corroborate and contribute to the existing body of research [31-34] by highlighting the significance of vessel density (VD) in the DCP as the most reliable OCTA parameter for discerning various stages of NPDR in young patients with type 1 DM. Previous studies have also indicated lower VD in the superficial capillary plexus (SCP) and DCP in eyes with retinopathy compared to healthy eyes. However, these earlier investigations were often smaller in scale and conducted on older individuals, many of whom had type 2 DM along with comorbidities. [30]

In the present study, there was no statistically significant difference in Choriocapillaris (CC) Vascular Density among the studied groups (p=.052).

Ryu et al. (2021),[35] demonstrated that the choriocapillaris vessel density also decreased with DR progression in the present study, and it was significantly associated with DR severity (p<.001 for all areas). In addition, they observed a significant association between DR severity and alterations in the macular microvasculature. Specifically, vessel densities in the SCP and DCP showed reductions across all macular regions even preceding the onset of DR.

Additionally, the vessel density of the choriocapillaris decreased in the perifoveal area before the onset of DR. Still, it was not significantly reduced in the foveal area until the PDR stage.

Choi et al. (2017),[36] observed CC alterations not only in cases of PDR and NPDR but also in individuals with diabetes who did not show signs of retinopathy. However, these alterations were generally less severe. This suggests that CC changes occur early in the course of diabetes and may contribute to DR. Further investigation into CC flow impairment at different stages of diabetic retinopathy is warranted to elucidate its significance.

Cao et al.'s (2018) [37] study revealed that individuals with type 2 DM who show no signs of DR exhibit decreased vessel density in the SCP, DCP, and choriocapillaris compared to those without diabetes. Furthermore, the study reported a significant association between vessel density in SCP and DCP and the duration of diabetes, levels of HbA1c, or serum creatinine.

Conti et al. (2019),[38] demonstrated that Eyes with NPDR and PDR showed significantly decreased choriocapillaris CPD compared with controls, while DM eyes without DR did not show significant change; however, they found no significant difference in choriocapillaris CPD between NPDR and PDR groups in this study. This finding may appear unexpected, considering that patients with PDR typically experience more severe ocular ischemia. However, a histopathological study by Cao et al.[39] assessed the degree of choriocapillaris degeneration in diabetic patients and demonstrated that eyes with severe chronic ischemia could develop Choroidal Neo-Vascularization (CNV).

CONCLUSION

Different OCT measurements, including Foveal Thickening, may be useful for the early detection of macular thickening and may be indicator(s) for a closer follow-up for diabetic patients.

Limitations

The small sample size is one of the limitations of the study.

Conflict of Interest

The authors declare no conflicts of interest.

Funding Statement

The authors received no specific funding for the conduction of this study.

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The Efficiency of Flexible Insulin using Carbohydrates Counting Program on Glycated Hemoglobin Test (HBA1C) Level in Diabetic Pediatric Patients in Tripoli.

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Received: 25/05/2024/ Accepted: 10/07/2024

ABSTRACT:

Background: The carbohydrate (CHO) counting program is used to adjust pre-meal insulin in intensive insulin regimens by using mathematical operations to convert grams of carbohydrates into equal units of insulin. However, diabetes mellitus children's patients in Libya are still treated with a fixed amount of insulin doses. CCP and determining the insulin dose after meals has proved its efficiency globally.
Objective: This study aimed to evaluate the effectiveness of flexible insulin using a carbohydrate counting program on the HbA1c level in pediatrics in Libyan hospitals.
Methods: Forty-five children aged between 2 and 18 from Aljala Children Hospital -Tripoli were observed for three months for their HbA1c which is the factor that is followed by doctors to determine the effectiveness of the insulin daily doses using CCP of their cases. This study was conducted to compare the level of HbA1c before the beginning of the program of carbohydrate counting and three months later. Affective factors may cause significant differences in the efficiency of CCP such as place of residency, BMI, gender, age, and other associated diseases. The collected data was statistically analyzed.
Results: The results indicate a notable reduction in HbA1c levels in children who adhered to the carbohydrate counting program (CCP). Additionally, attention was given to side effect factors to understand their impact on HbA1c levels and how they are interrelated. Factors like weight and insulin doses were found to have a significant influence, as the response to the CCP program varied among cases based on these factors.

Keywords: diabetes, blood glucose, dietary carbohydrates, medical nutrition therapy, child Component; Formatting; Style; Styling; Insert (keywords)

INTRODUCTION

Diabetes Mellitus (DM) is a chronic endocrine disorder that causes high blood sugar levels due to problems with insulin secretion or action. It's the most common chronic endocrine disorder among children. Despite efforts to reduce blood sugar levels, diabetes remains incurable, and its prevalence is increasing worldwide, especially in low- and middle-income countries like Libya. In Libya, over 70% of the population is affected by diabetes, which is the highest prevalence in North Africa and among Arabic nations (1). Type 1 diabetes (T1D) affects children and young individuals and has been linked to cardiovascular diseases and higher mortality rates. Managing T1D and lowering the risk of complications requires maintaining optimal blood glucose levels. Unhealthy lifestyles, such as being overweight and obese, are prevalent among individuals with T1D, contributing to cardiovascular risks (1,2)

The management of Type 1 diabetes has improved over the years, especially in children, with the introduction of new insulin strategies that allow better control of blood sugar levels. However, fluctuations in glycemic levels, particularly after meals, are still associated with cardiovascular complications. To prevent these issues, it is essential to manage postprandial glucose spikes, which rely on well-designed insulin therapy regimens and carbohydrate counting (3).

Carbohydrate counting is a common practice for T1D patients, which helps match insulin dosage with the carbohydrate content of meals. However, it has its limitations, and the introduction of continuous glucose monitoring has revealed the impact of other nutrients, such as fat and protein, on postprandial glucose response. Adjusting insulin delivery strategies based on these nutrients has not yielded conclusive results, and the glycemic index of foods further complicates the management of postprandial blood glucose response (23).

Diabetes is a serious disease that cannot be cured and has significant consequences. To prevent cardiovascular complications, it's crucial to manage postprandial glucose levels by considering carbohydrate counting and nutrient quality (22). However, developing effective therapeutic strategies is challenging due to the complex nature of individual responses and interactions between nutrients (11).

Carbohydrate counting program CCP:

Carbohydrate counting is a popular method used by individuals with type 1 diabetes (T1D) to match their insulin dosage with the amount of carbohydrates in their meals. However, this method has some limitations. The introduction of continuous glucose monitoring has exposed flaws in carbohydrate counting and has highlighted the impact of other nutrients, such as fat and protein, on postprandial glucose response in T1D. Altering insulin delivery strategies based on these nutrients has not yielded conclusive results, and the recommended increase in insulin-to-carbohydrate ratio for fat and protein varies among studies. Furthermore, it is important to consider the energy content of meals, as this can affect postprandial glucose response in real-life situations. The quality of nutrients, particularly the glycemic index of foods, further complicates the management of postprandial blood glucose response. High glycemic index meals cause a rapid increase in glucose levels, followed by a quick decline, which increases the risk of hypoglycemia. On the other hand, low glycemic index meals lead to a delayed rise in blood glucose levels (10,11).

It can be challenging to incorporate carbohydrate quality into algorithms for pre-prandial insulin dosing. This is due to the significant variability in glucose response between individuals and the difficulty in accurately considering the glycemic index of

individual foods and mixed meals. Even with advanced technologies, managing high glycemic index meals remains difficult, mainly because insulin absorption is slower compared to simple sugars. The quality of fat also affects postprandial glucose response. For example, using extra-virgin olive oil instead of butter resulted in a blunted early glucose response to a high glycemic index meal (18).

The current methods used to manage postprandial glucose variability in individuals with type 1 diabetes are still not sufficient due to the complex interactions between nutrients and various factors that affect individual metabolic responses. The significant variation in responses among individuals poses a challenge. Recent studies suggest that the composition of microbiota may play a role in influencing unique reactions. Therefore, future studies should focus on developing personalized strategies to effectively manage fluctuations in postprandial glucose levels among individuals with type 1 diabetes (17).

♦ **Study objectives:**

1-To determine the efficiency of implementing CCP and using flexible insulin doses to decrease the HbA1c levels in Type 1 diabetic children in Libya.

2-To evaluate the outcomes of CCP on HbA1c levels.

3-To educate patients on how to adjust their insulin dose based on their carbohydrate intake.

4-To identify the factors that may influence or affect the results of CCP program.

MATERIALS AND METHODS

This is a prospective cohort observational study that screened and assessed randomized cases of interventions. The trial lasted longer than three months and aimed to compare a carbohydrate counting program (CCP) with general or alternate dietary advice in 45 diabetic children aged 2-15 years who were diagnosed with type 1 diabetes. The primary outcome was the change in glycated hemoglobin (HbA1c) concentration. The patients who were in regular contact with the nutritionist were counted as qualified cases. The study used a comprehensive questionnaire as a tool, and all patients were diagnosed with type 1 diabetes and used the Flexible Insulin with Carb Counting Program (CCP) between October 2019 and November 2021. The study was conducted at Aljala Children Hospital in Tripoli, Libya (15,16).

The HbA1c level is a meter that doctors use to measure average blood sugar levels over weeks or months, usually three months. This parameter is used to evaluate the effectiveness of the CCP (1,2). To measure HbA1c, doctors use a capillary method using the Nycocard II Reader device, which is suitable for the International Federation of Clinical Chemistry (IFCC) Working Group reference system. The study compared the rate of HbA1c before the program began and three months later to determine its effectiveness. The study aimed to distinguish the effect of several factors that influence HbA1c levels, such as the incidence of other diseases, place of residency (inside or outside Tripoli or distance from the hospital), body weight presented by MBI, gender, and age of patients. The HbA1c level is a meter that doctors use to measure average blood sugar levels over weeks or months, usually three months. This parameter is used to evaluate the effectiveness of the CCP (1,2). The study compared the rate of HbA1c before the program began and three months later to determine its effectiveness. The study aimed to distinguish the effect of several factors that influence HbA1c levels, such as the incidence of other diseases, place of residency (inside or outside Tripoli or distance from the hospital), body weight presented by MBI, gender, and age of patients (16).

Permission was obtained from the relevant authorities to collect data for this study. The data collected only included cases that matched the study's criteria. To ensure accuracy,

patients were closely monitored by their doctors and nutritionists to track their carbohydrate (CHO) consumption during treatment. Although various methods for counting carbohydrates are used in pediatric practice, there are two important measurements that patients should consider while following the Carbohydrate Counting Plan (CCP):

1. Carbohydrate coverage insulin at a meal: To calculate the carbohydrate coverage insulin dose, use the following formula: CHO insulin dose = Total grams of CHO in the meal ÷ grams of CHO disposed of by 1 unit of insulin (the grams of CHO disposed of by 1 unit of insulin is the denominator of the Insulin: CHO ratio).

2. High blood sugar correction dose: To calculate the high blood sugar correction dose, use the following formula: High blood sugar correction dose = (Actual blood sugar - Target blood sugar) ÷ Correction factor (6.14).

Food products are required to have information about their carbs written on their labels. However, whole foods like fruits and vegetables do not have labels. To calculate the carb amount in such foods, there are various apps and tools available online. During a training session at Aljala Hospital, 11 patients were observed and evaluated before and after using CCP to determine their HbA1c levels. The results showed that three patients did not see any improvement, while 60% showed improvement. This led to further study to evaluate the effectiveness of the CCP.

Statistical analysis:

A statistical analysis was conducted to evaluate the impact of various factors, both individually and collectively, on HbA1 levels. Doctors selected HbA1c as the primary measure to assess the effectiveness of the flexible insulin program with CCP through close monitoring of cases. The calculation process took into account the variation in blood sensitivity across different cases (2,3). The initial data was entered into Excel spreadsheets. Descriptive statistics such as frequency distribution, mean, and standard deviation were utilized to analyze the data. To assess statistically significant differences, one-way ANOVA and multivariate ANOVA analyses were performed using Excel 2016. Variances were compared at a 95% confidence level by employing an F-test with a p-value = 0.05.

RESULTS

1. **Participation analysis:**

1.1 The age groups of the participated cases: The prevalence of diabetes among the age group of 6-18 in Libya is alarmingly high, as depicted in Figure (1).

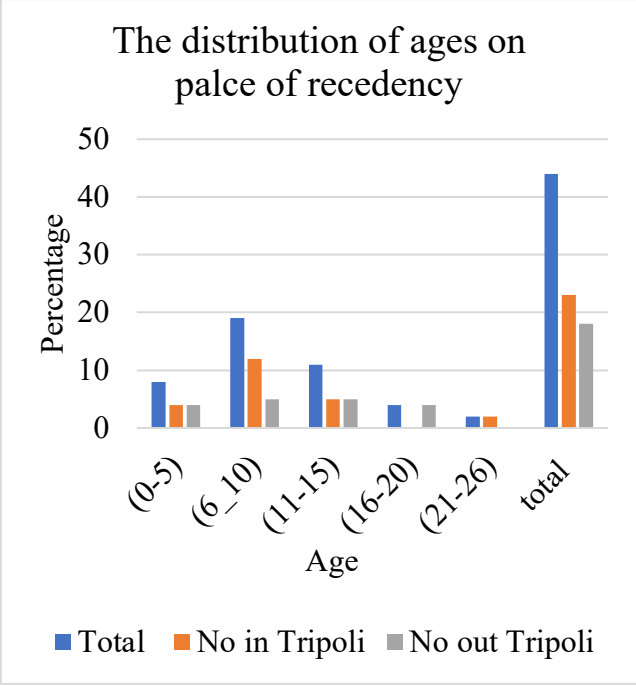

Figure (1). The effect of age on the diabetes distribution among children.

2. Factors influence the CCP efficiency:

2.1 The effect of age and gender on the efficiency of CCP: Figure (2) shows that males are more susceptible to elevated Hbc1A levels across all age groups. Male teenagers, in particular, had a Hbc1A level of 12.3 before the trial, which decreased to 8.4 after three months of insulin treatment using the CHO counting strategy. In Table (1), it was observed that before implementing CHO counting, Hbc1A levels were highest among girls aged 6-10. However, among boys aged 11-15, Hbc1A levels were highest. The study found that there is a correlation between age and gender on HbA1c levels, where males consistently have higher levels than females across all ages

Table (1). The correlation between age and gender on the accumulative Hbc1A levels before and after CCP.

Years	HbA1c (1)F	HbA1c (1)M	HbA1c (2)F	HbA1c (2)M
0-5 yr.	9.65[a]	8.25[a]	6.73[b]	7.57[a]
6-10 yr.	10.5[a]	9.06[a]	7.53[b]	7.3[b]
11-15 yr.	10.1[a]	12.3[c]	7.68[b]	8.4[b]
16-18 yr.	8.86[b]	10.4[a]	6.86[b]	7.65[b]

Different letters in the same row indicate statistically significant differences at ($P < 0.05$).

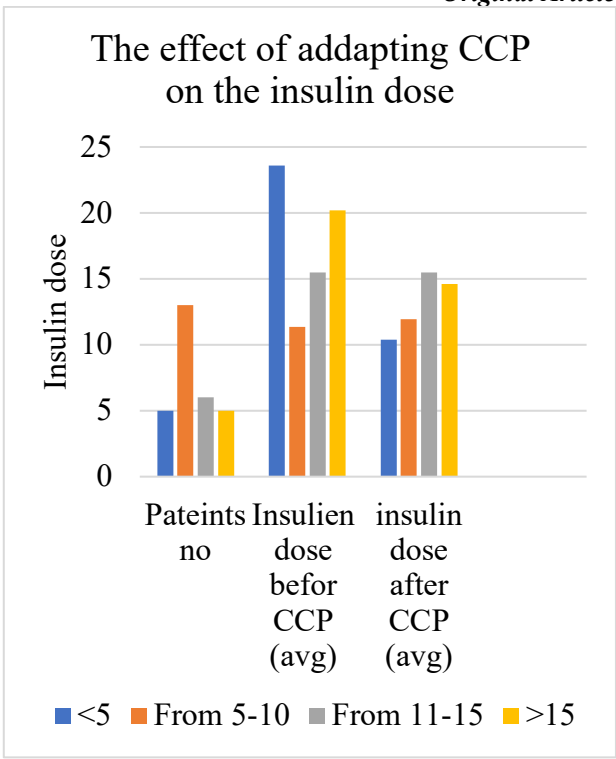

Figure (2). The effect of CCP on insulin doses in different ages.

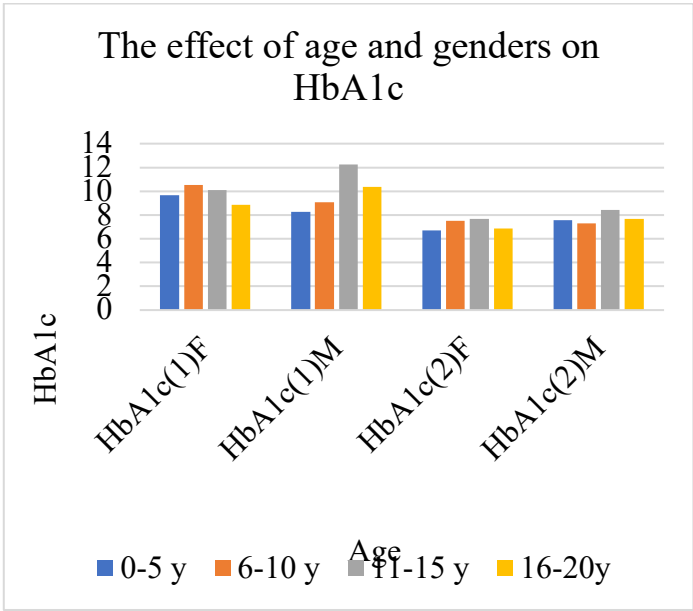

Figure (3). The effect of age and gender on Hbc1A levels. (1= before CCP,2=after CCP), F= female, M= male.

Table 2: Clinical and laboratory outcomes of the patients when using standard insulin dosing before carbohydrate counting and when using flexible insulin dosing according to carbohydrate counting.

Parameters	Standard insulin dosing before carbohydrate counting	Flexible insulin dosing according to carbohydrate counting	P value
BMI (kg/m^2)	22	22	0.184
Hemoglobin A1c (%)	8.2	7,3	0.006
Fasting plasma glucose (mg/dL)	165.7	140.7	0.048
Post-prandial glucose (mg/dL)	231.1	149.43	0.001
Triglyceride (mg/dL)	85	93	0.752
HDL-cholesterol (mg/dL)	55.5	65.5	0.038
LDL-cholesterol (mg/dL)	85.5	65.5	0.035
Hypoglycemia (episodes/month)	4	4	0.113
Total insulin dose (IU/day)	38.5	37	0.727

2.2- The effect of weight, presented by body mass index (BMI) parameter, on HbA1c: The results indicate that for females, the P-value for the comparison of BMI between HbA1c levels before and after CCP was 0.45. Since this value is greater than 0.05, the effect of BMI on CCP results was insignificant. Similarly, for males, the P-value was 0.184, which is also greater than 0.05, suggesting that the effect of BMI on CCP results was not significant in males. Therefore, the null hypothesis (H0) is not rejected for both genders. Additionally, Post-prandial plasma glucose was 231.10 mg/dl before and 149.43 mg/dl after carbohydrate counting (P = 0.001). LDL-cholesterol levels were 85.50 mg/dl when patients used a standard insulin dose and 78.50 mg/dl when they used flexible insulin dosing (P = 0.035). HDL-cholesterol levels were 55.50 mg/dlbefore carbohydrate counting; they reached 65.50 mg/dl with flexible insulin dosing (P = 0.038). However, a statistically significant difference was not detected between the periods before and after carbohydrate counting regarding basal insulin dose, bolus insulin dose, triglyceride level, body mass index,or monthly hypoglycemia episode count (P > 0.05) (figure 4, table 2).

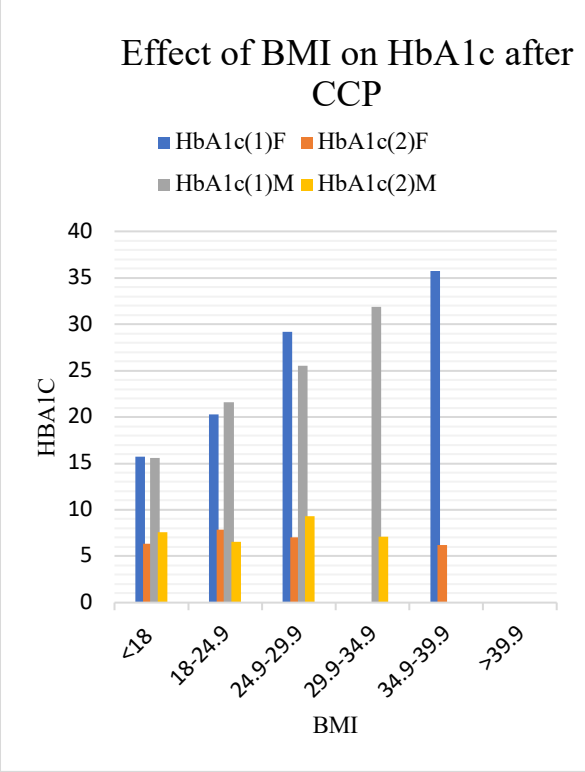

Figure (4). The effect of BMI on Hbc1A after CCP. (1= before CCP, 2=after CCP)

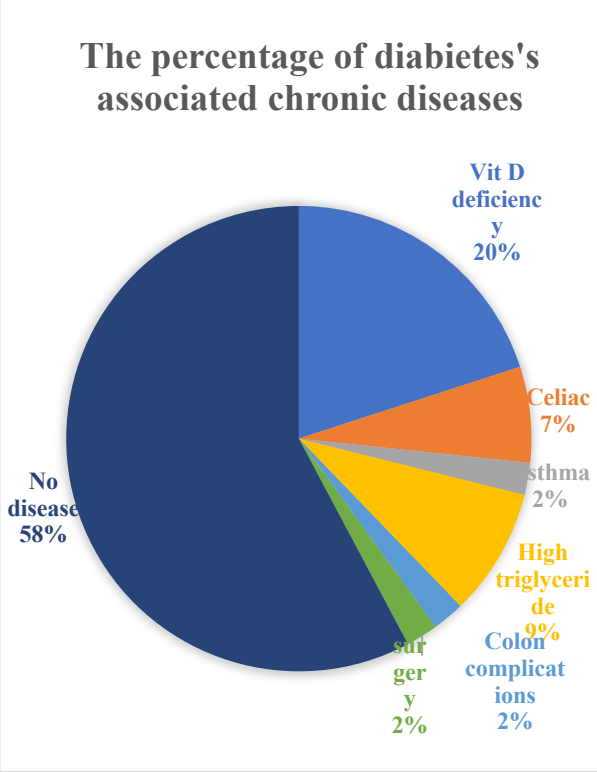

Figure (5). The percentages of other associated diabetes diseases.

3- Diabetes associated diseases:

The study also found that a majority of patients experienced a significant decrease in vitamin D levels (58%), and high percentages of children had high triglyceride levels (9%) and Celiac disease (7%). The study also showed that many patients were younger at the time of diagnosis, which also had an impact on the HbA1c levels (Figures 5 & 6).

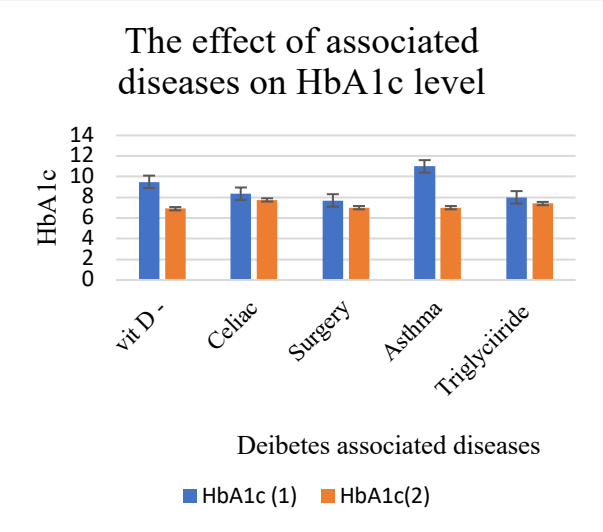

Figure (6). The effect of associated diseases on HbA1c level. (1= before CCP, 2=after CCP).

4-The effect of adapting CCP on HbA1 levels in all cases: Figure (7) demonstrates the impact of the carb counting program on HbA1c levels in various cases. The analysis of results showed that the difference was statistically significant, with a P-value of 0.58, less than 0.05. This led to a significant decrease in HbA1c levels after the adoption of the CCP program.

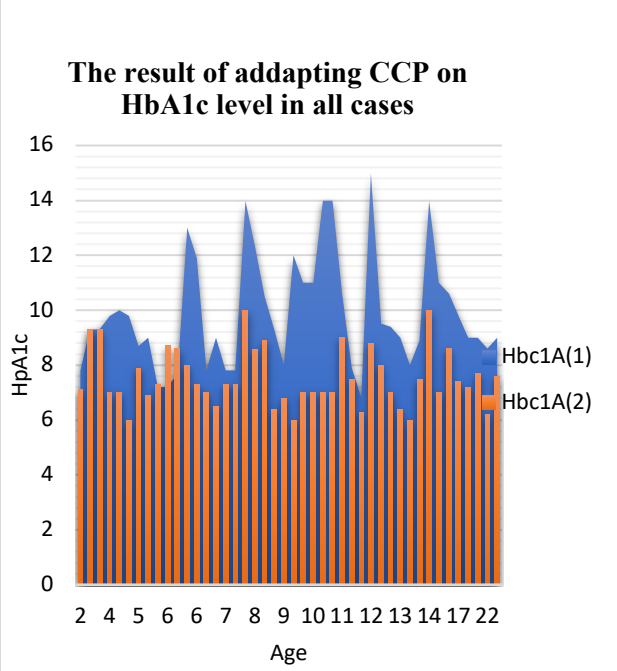

Figure (7). The effect of adapting CCP on HbA1c levels in all cases. (1= before CCP, 2=after CCP.

DISCUSSION

In this study, the prevalence of diabetes among the age group of 6-18 in Libya is alarmingly high. This raises concerns about the health and well-being of the younger population. The concentration of diabetes cases in Tripoli suggests that urban areas face a greater burden in terms of this chronic disease.

The finding that a significant number of study participants were younger than 6 years old is intriguing and points towards a recent increase in diabetes cases. This trend could be attributed to the detrimental effects of an unhealthy lifestyle and malnutrition that children have experienced over the past decade. The ongoing wars and political conflicts in Libya have significantly impacted the overall living conditions of the population, including access to nutritious food and healthcare services. The conflicts in Libya have had a direct impact on the economic circumstances of the population, resulting in many people leading a sedentary lifestyle. Limited economic opportunities and instability have contributed to a lack of physical activity and an increased reliance on unhealthy food options such as fast food, sweets, and desserts. This situation has been further exacerbated by the fast-paced nature of modern life in Libya, where people increasingly rely on eating outside of their homes due to time constraints and convenience. Unfortunately, this trend has led to the uncontrolled intake of calories by children outside their homes, which has become a contributing factor to the development of diabetes.

Addressing the increasing prevalence of diabetes among Libyan children and adolescents requires a multifaceted approach. This includes promoting healthier lifestyles, improving access to nutritious foods, and enhancing healthcare services. Education and awareness campaigns can play a crucial role in informing the population about the risks associated with an unhealthy diet and sedentary behavior. Additionally, efforts should be made to create environments that support physical activity and provide opportunities for children to engage in regular exercise.

Analysis shows that males are more susceptible to elevated Hbc1A levels across all age groups. Male teenagers, in particular, had a Hbc1A level of 12.3 before the trial, which decreased to 8.4 after three months of insulin treatment using the CHO counting strategy. This could be due to two potential reasons. Firstly, males may have a genetic predisposition to developing diabetes at an earlier age than females. Secondly, it could

be a result of males adopting unhealthy eating habits during their teenage years. These findings are consistent with previous studies that support the same theory.

In our finding, it was observed that before implementing CHO counting, Hbc1A levels were highest among girls aged 6-10. However, among boys aged 11-15, Hbc1A levels were highest. This can be explained by the fact that females in this age group are more likely to follow their parents' instructions compared to males in Libya. Despite this result, another study conducted in Benghazi in 1996 revealed a higher incidence of type 1 diabetes in females aged 0-14 and a higher incidence of type 2 diabetes in females aged 15-34. In both cases, males had a lower incidence rate (18).

The study found that there is a correlation between age and gender on HbA1c levels, where males consistently have higher levels than females across all ages. One possible explanation for this is that females at this age are more likely to follow their parents' instructions. Further statistical analysis showed that there was no statistically significant difference between females before and after the CCP program, with a p-value of 0.45., indicating no statistically significant difference.

A study conducted in Australia aimed to evaluate the outcomes of a program designed to teach diabetic patients how to adjust their insulin dose based on their carbohydrate intake, flexible eating, and flexible insulin dosing. The study observed 40 individuals with type 1 diabetes who adopted this program and focused on monitoring their HbA1c levels. Sixteen individuals who did not fully embrace intensive insulin management and twenty-four individuals who initially had an HbA1c level below 7% were excluded from the analysis. The study's intention-to-treat analysis revealed that the participants' average HbA1c levels decreased from 8.7% at the beginning of the study to 8.1% after 12 months. Furthermore, the number of individuals with an HbA1c level below 8% increased from 67 (48.9%) before the program to 86 (62.8%) afterward. These findings suggest that the intensive diabetes self-management program using CCP led to improvements in HbA1c levels, empowerment, and quality of life, which were largely sustained at the one-year mark (12).

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In 2010, Rossi conducted a study that showed the advantages of flexible eating and insulin dosing for diabetic patients who took part in an intensive self-management program. The study found that consistent carbohydrate intake, both in terms of amount and source, was linked to better blood glucose control in individuals with type 1 diabetes (19).
Various studies have shown that advanced CCP implementation has led to a positive trend in HbA1c improvement. The reduction in HbA1c levels ranged from 0.0 to 13 mmol/mol (0.0–1.2%), and most measures of psychosocial well-

being improved, although only a few improvements were considered clinically significant. In some cases, weight gain and weight loss were observed, but most studies did not find any significant weight changes. The majority of studies that assessed the occurrence of hypoglycemic events reported a significant reduction in the event rate, and none of the studies reported an increase in incidence (7,8,9).

The results indicate that for females, the P-value for the comparison of BMI between HbA1c levels before and after CCP was 0.45. Since this value is greater than 0.05, the effect of BMI on CCP results was not significant. Similarly, for males, the P-value was 0.184, which is also greater than 0.05, suggesting that the effect of BMI on CCP results was not significant in males. Therefore, the null hypothesis (H0) is not rejected for both genders. To conclude, the CCP has a significant impact on weight gain and vice versa, as shown by a study conducted in Benghazi in 1996, where 56% of diabetes mellitus (DM) cases were obese, compared to only 6% of DM cases (Kadiki 1996). Statistically, the P-value for BMI was calculated as 0.0885, which is larger than 0.05, indicating that BMI may affect the effectiveness of CCP. Additionally, the P-value for the effect of BMI on HbA1c was 0.318, which is also larger than 0.05. Hence, the CCP may lead to weight gain in patients (4,10). In our study, a statistically significant difference could not be detected about basal and bolus insulin doses or some hypoglycemic episodes when treatment was switched to flexible insulin dosing from standard insulin dosing ($P > 0.05$). Daily total insulin dose decreased by 3.8% ($P = 0.752$), bolus insulin dose decreased by 5.2% ($P = 0.224$), and basal insulin dose increased by 25% ($P = 0.055$). The monthly median hypoglycemic episode count was equal in both conditions at 4 hypoglycemic episodes/month. HbA1c levels would be normal in all diabetic patients if hypoglycemia did not occur. Hypoglycemia limits the long-term benefits of glycemic control in patients with type 1 DM Flexible insulin dosing, carbohydrate counting, and flexible eating provide better glycemic control without increasing hypoglycemia risk in patients with type 1 DM (18,19).

According to a recent study, certain factors can impact HbA1c levels, including inflammation in the body, which can cause an increase in HbA1c rates. Due to this, it can be challenging to accurately interpret the results. To address this, researchers looked at each patient's clinical history to evaluate how other diseases might affect their response to medication. Allergies and chest infections are two examples of diseases that can be influenced by cortisone. The study also found that a majority of patients experienced a significant decrease in vitamin D levels (58%), and high percentages of children had high triglyceride levels (9%) and Celiac disease (7%). The study also showed that many patients were younger at the time of diagnosis, which also had an impact on the HbA1c levels. In a study conducted by Ashabani in 2003 in Tripoli, it was discovered that CD was more prevalent among Libyan children compared to several European countries (1,2).

The analysis of results showed that the difference was statistically significant, with a P-value of 0.58, higher than 0.05. This led to a significant decrease in HbA1c levels after the adoption of the CCP program. The results were consistent with various studies that have shown the effectiveness of the CCP in promoting change, especially in patients with poor schooling. Group care can improve the quality of life of individuals with type 1 DM. The study suggested that specific educational and psychological supports are necessary to modify adaptation to the CCP (3, 5, 24).

Diabetes presents unique challenges in sub-Saharan African populations, including early onset, limited understanding of specific types such as ketosis-prone atypical diabetes, high rates of undiagnosed cases, and an increased risk of chronic complications. Therefore,

implementing a comprehensive and culturally sensitive approach to diabetes control and care is essential to reduce the burden of the disease and improve health outcomes in this region (15).

In our study, a statistically significant difference could not be detected in basal and bolus insulin doses or some hypoglycemic episodes when treatment was switched to flexible insulin dosing from standard insulin dosing ($P > 0.05$). Daily total insulin dose decreased by 3.8% ($P = 0.752$), bolus insulin dose decreased by 5.2% ($P = 0.224$), and basal insulin dose increased by 25% ($P = 0.055$). The monthly median hypoglycemic episode count was equal in both conditions at 4 hypoglycemic episodes/month. HbA1c levels would be normal in all diabetic patients if hypoglycemia did not occur. Hypoglycemia limits the long-term benefits of glycemic control in patients with type 1 DM Flexible insulin dosing, carbohydrate counting, and flexible eating provide better glycemic control without increasing hypoglycemia risk in patients with type 1 DM (18,19).

CONCLUSION

The findings of this study show that the CCP program is an effective approach to reducing HbA1c levels in a majority of children assessed at the diabetes center in Tripoli. The program aims to educate parents on adopting healthy lifestyles and eating habits to achieve optimal glycemic control, maintain a healthy body weight, and prevent various complications associated with diabetes such as hypoglycemia, hyperglycemic crisis, and diabetes-related diseases that affect small and large blood vessels, the eyes, kidneys, nerves, heart, and brain vessels. Furthermore, regularly monitoring blood sugar levels and discussing them with a healthcare provider can provide valuable insights into how the CCP program's meal plans can affect glucose and HbA1c levels. In summary, it is recommended to implement a flexible insulin regimen by adopting the carbohydrate counting program for all diabetes patients in Libya as it significantly reduces HbA1c levels. To conclude, policymakers and healthcare professionals need to work together and develop effective strategies that tackle the root causes of diabetes in Libya at an early age. By prioritizing prevention, early detection, and management, we can reduce the negative impact of diabetes on the health and wellness of young people in Libya.

ACKNOWLEDGEMENT

Ethical considerations

The study was approved by the Department of Medical Laboratories Sciences at Tripoli University. All participants signed consent before participating in the study.

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Prevalence of Toxoplasmosis Among Pregnant Women in Benghazi City / Libya

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Received: 02/06/2024 Accepted: 12/06/2024

ABSTRACT:

Toxoplasmosis is caused by *Toxoplasma gondii*, an obligate intracellular parasite that infects both humans and animals as a zoonotic pathogen widespread in nature. This study aims to determine the prevalence of toxoplasmosis among pregnant women in Benghazi City and identify risk factors that affect the infection rate. A total of 227 women who attended different private medical laboratories in Benghazi City to perform toxoplasma screening tests during pregnancy were selected. The duration of the study was from January 1, 2023, to October 1, 2023. In addition, patients were examined for antibodies to *Toxoplasma gondii* (Ig G) and (Ig M) by the commercially available technique of ELISA. To be more specific, five age groups were used. Consequently, the total positive infected pregnant women were 44.1%, besides the negative non-infected women, who were 55.9%. The seroprevalence of active toxoplasmosis was 44.1%, and chronic infections were 30%. The patient's age had no significant relationship with the T. *gondii* infection. To conclude, with the publication of health education and disease definitions being recorded, infection will be reduced among pregnant women who are more susceptible to Toxoplasma infection. Therefore, pregnant women should be screened for toxoplasmosis regularly. This study aims to evaluate the seroprevalence of *T. gondii* infection among pregnant women of various age groups and to identify associations between infection and certain risk factors for infection.

KEYWORDS: Toxoplasmosis; Toxoplasma gondii; pregnant women; Benghazi; Libya.

INTRODUCTION

Toxoplasmosis is caused in humans and other vertebrate species by *Toxoplasma gondii*, an apicomplexan protozoan parasite with a heterogeneous life cycle, and the disease is very prevalent in humans around the world (1). In addition, the only known definitive host for *T.gondii* is a member of the Felidae family, which includes domestic cats and their relatives. This coccidian parasite can infect humans and warm-blooded animals as intermediate hosts (2). Significantly, humans can be infected with *T.gondii* by four major pathways: ingesting contaminated food or water by infected cat oocysts; ingesting or handling undercooked or raw meat containing tissue cysts; receiving organ transplants or blood products from donors with acute or latent toxoplasmosis; or, congenitally, through transplacental transmission of tachyzoites (3). Most human infections are asymptomatic. However, the parasite can often produce symptomatic disease with severe or even fatal clinical outcomes, especially in infected fetuses and immunodeficient individuals (4). T. gondii infection in fetal and neonatal animals may result in death or numerous congenital defects during pregnancy, such as hydrocephalus, disorders of the central nervous system, and chorioretinitis. Also, it can cause central nervous system manifestations such as Guillain-Barré syndrome (5) or cause brain abscesses in immunocompromised patients. Human toxoplasmosis prevalence in North African countries causes severe morbidity and financial losses. Thus, toxoplasmosis poses a serious risk to human and animal health. (10) released a review paper on toxoplasmosis and T. gondii infections in North Africa, affecting both humans and animals. The research was conducted in five North African nations: Morocco, Algeria, Tunisia, Libya, and Egypt. In Morocco, seroprevalence was between 36.7% and 62.1% in 2007 and 2017, respectively. Conversely, a serological test conducted in Algeria revealed that between 30% and 53.2% of people had T. gondii antibodies. Moreover, ELISA results show seroprevalence rates vary from 39.3% in the southern regions to 47.7% in the northern parts of Tunisia. Whereas, in Tripoli, Libya, 11 reported a 38.5% infection rate among women. Nationally, there aren't many studies on toxoplasmosis in Libya. A few recorded investigations on the prevalence rate of *T. gondii* in Libya were published by (6), who found that the disease affected 51.6% of adult males, 43.4% of adult females, 43.7% of schoolchildren, 18.14% of non-pregnant women, and 17.6% of women who reported spontaneous abortions. Toxoplasmosis in some cities near Benghazi City is more recent and updated, such as one study published by (7), who noted that the seroprevalence of IgG of *T. gondii* in El-Beida City is 26.86%, indicating a chronic infection. In contrast, the seroprevalence of IgM of *T. gondii* is 11.94%, considering it a new infection. In Sirte, Libya, (8) reported the toxoplasma infection rate was 35.92 % in males and 55.67 % in females. Finally, (9) claims that overall IgG seropositivity rates were detected in women with previous adverse pregnancies to be 44.8% in Benghazi city. Since approximately many people are thought to be chronically infected with *T. gondii,* toxoplasmosis is a major health concern not only in Libya but also in many parts of the world. In reality, several diagnostic techniques can be used to identify a human infection with *T. gondii*; the most significant of these is ELISA, which is one of numerous serological techniques to identify various antibody types (12). Regarding the formation of antibodies against *T. gondii,* IgM antibodies can be found within a week after the infection and will likely remain for months. Consequently, establishing an acute infection will require the detection of IgM. In contrast, the presence of IgG antibodies in infected patients can persist for many years. Therefore, it indicates an old infection (12). The purpose of this study was to ascertain the *T. gondii* seroprevalence of IgG and IgM among pregnant women in Benghazi city, Libya, as there is insufficient information

regarding the prevalence of toxoplasmosis in that city.

MATERIALS AND METHODS

Study area: This study was conducted in Benghazi City, Libya. With an estimated population of 632,937 in 2019, Benghazi is the second-most populous city in Libya and is located on the Gulf of Sidra in the Mediterranean.

Study Samples: The population of this study was 227 pregnant women, recorded from many different private clinics. The sample ages were broken down into five age groups (group 1: 20–29 years, group 2: 30-39 years, group 3: 40–49 years, group 4: 50–59 years, and group 5: 60–65 years).

Collection of blood samples: about 5 ml of venous blood was collected under sterile conditions from each individual by medically trained nurses at the related clinics. After that, it was transported to the microbiology laboratory, where the blood samples were centrifuged at 4000 rpm for 5 minutes to separate the serum. Finally, analysis was completed using the ELISA (enzyme-linked immunosorbent assay) technique.

Data analysis was performed with SPSS (statistical package for social science) version 25, 1989, 2017; statistical significance was taken at $p \leq 0.05$.

RESULTS AND DISCUSSION

The current study is one of a series of studies in Libya that assess some behavioral factors that may influence the rate of *Toxoplasma gondii* infection in Benghazi and Libya.

The toxoplasma infection rate: It was found that 100 sera blood samples (44%) out of 227 tested were positive for toxoplasma gondii infection, while 127 sera blood samples (56%) were negative for *T. gondii* infection, as shown in Figure 1. The result was statistically significant ($P < 0.05$).

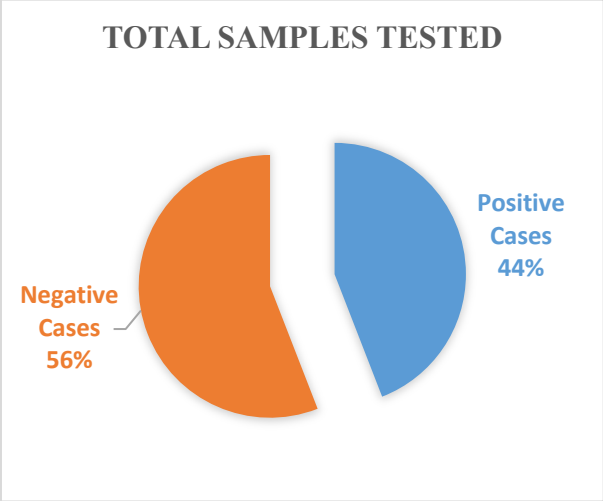

Figure 1: The prevalence of *T. gondii* (positive and negative samples) *infection* rate among total tested samples.

Percentage of IgG and IgM Toxoplasma gondii infection-positive cases: Out of 227 sera blood samples collected from pregnant women, a total of 86 (38%) and 14 (6%) IgG and IgM, respectively, were found to be seropositive for antibodies against *T. gondii* infection as shown in table 1 with a p-value of ($P < 0.05$).

Ig class	Total No. of samples	Total No. of positive samples & (%)
Ig G	227	86 (38) %
Ig M	227	14 (6) %

Table 1: Percentage of IgG and IgM Toxoplasma gondii infection-positive cases.

Relationship between the toxoplasma infection rate and age groups: It was clear from the present results shown in Table 2 that the sera blood sample showed an increase in the percentage of anti-*T. gondii* IgM within the age

group 40–49 years (9,64%), in addition, to an increase in the percentage of anti-*T. gondii* IgG (28,33%). However, the age group between 60 and 65 showed the lowest percentage of anti-*T. gondii* IgM and IgG, being 17% and 33%, respectively. Therefore, there was an association between the prevalence of infection (regarding whether it was a chronic or active infection) and age group, as shown in Table 2. Toxoplasmosis is recognized as one of the most significant members of the TORCH group, which also includes the most frequent infections linked to medical issues during pregnancy, including cytomegalovirus, herpes, and rubella (1). Furthermore, spontaneous abortion has been linked to primary maternal *T. gondii* infection throughout pregnancy (1, 2). The frequency of *T. gondii* among expectant mothers has been thoroughly investigated and recorded in several Libyan cities; these studies revealed notable regional variations in the infection's prevalence (3, 4).

Conversely, ELISA is the most widely used test to identify T. gondii antibody classes (1) serologically. This study's data, based on the ELISA technique, was collected retrospectively from different private medical laboratories in Benghazi, Libya, in 2023. The target subjects were pregnant women who were suspected of having a *T. gondii* infection. 100 positive samples were recorded out of 227 samples (44%), and 127 samples were negative for *T. gondii* infection out of 227 samples (56%), as shown in Figure 1. The current results were not very different from those observed in other previous studies from different regions of Libya. In Tripoli, 45% of seropositives for *T. gondii* infection were reported by (5). Another study, which was done on blood donors, found that 33.5% of blood donors had a T. *gondii* infection (6). In Sirte city, (7) claimed that *T. gondii* infection was positive for 45.5 percent of cases, while 54.50% were negative cases. Furthermore, there was a seroprevalence study of toxoplasmosis among Libyan pregnant women in Benghazi city, as (44.8%) of *T. gondii* IgG seropositive and (8.4%) of *T. gondii* IgM seropositive (8). These results matched the findings of the current investigation, which showed that *T. gondii* IgM was detected in 14 positive samples out of 227 (6%) with a p-value of 0.000. On the other hand, 86 out of 227 samples (38%) tested positive for *T. gondii* IgG, with a 0.000 p-value. Although these results are consistent with those of previous studies conducted in several Libyan locations, other regions of the country have low incidence rates. For example, one study, which was conducted in Benjawad, Libya, claimed that anti-*T. gondii* IgG and IgM antibodies were found to be positive in 37.14% and 3.57% of pregnant women, respectively(9). One further study was conducted in the Sebha region, which found that 36.84% of the sera from 190 pregnant women tested positive for anti-*T. gondii* IgG (10). Therefore, some reasons could be responsible for the differences and similarities in Toxo IgG and IgM seroprevalences from region to region within Libya, such as contact with animal species, the existence of cats on farms, drinking untreated water, becoming older, being in a climate that isn't too hot or cold, and cultural differences regarding the practice of food consumption and sanitation customs (5, 13). Even though the previous reasons may contribute to the toxoplasmosis incidence in Libya, the environmental conditions are more likely to be the reason for the fluctuating incidences in different parts of Libya.

According to age group, the serum blood samples revealed an increase in *anti-T. gondii* IgM percentage (9%) and *anti-T. gondii* IgG percentage (28%) in the 40–49 age group. Nevertheless, the age group ranging from 60–65 had the lowest levels of *anti-T. gondii* IgM and IgG, at 1% and 3%, respectively (shown in table 2). Therefore, age and seropositivity in this study showed a strong connection, suggesting that older populations are more vulnerable to the parasite than other age groups. These findings are consistent with those of (11) who found that infection rates rose with age and that the greatest incidence of infection was (69.3%) in the 46–50 age group.

Table 2: The Relation between T. gondii-specific IgG and IgM and the Age of Tested Samples

		Total No. of samples & %	No. of positive IgM & % Out of 14	No. of positive IgG & % Out of 86
Age groups	20-29	71 (31.3%)	4 (29 %)	16 (19 %)
	30-39	74 (32.6 %)	0 (0%)	18 (21 %)
	40-49	55 (24.2 %)	9 (64 %)	28 (33 %)
	50-59	21 (9.3 %)	0 (0%)	21 (24 %)
	60-65	6 (2.6 %)	1 (7 %)	3 (3%)
Total		227	N = 14	N = 86

CONCLUSION

This study confirmed that pregnant women are a risk group for toxoplasmosis. The publication of health education and disease definitions will reduce the incidence of toxoplasmosis among pregnant women in the coming years. It is highly recommended for pregnant women to be screened for toxoplasmosis to prevent any side effects that may occur during the pregnancy period.

In addition, blood donors should be screened for toxoplasmosis since pregnant women may be transfused by blood that contains the effective stage of the parasite.

Conflict of interest

The author declares that he has no conflict of interest.

Acknowledgment

I acknowledge everyone who assists and supports this scientific work.

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Prevalence, Epidemiological Pattern and Causative Pathogen of Urinary Tract Infection Among Children Admitted to the Pediatric Department of Tobruk Medical Center

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Received: 02/06/2024 Accepted: 11/06/2024

ABSTRACT:

Background: Urinary tract infections (UTIs) are common in pediatric populations and are among the most prevalent bacterial diseases worldwide, particularly in infants and young children, making them a significant pediatric health concern[1]. UTIs can manifest with varying degrees of severity and can be caused by a spectrum of pathogens. UTIs are particularly important to address because when they affect the upper urinary tract, they can form scars that damage the growing kidney and predispose individuals to hypertension and chronic kidney disease [3].

Patients and Method: To estimate the prevalence of urinary tract infection (UTI) and to determine the causative pathogens among children admitted to the pediatric department at Tobruk Medical Center, a retrospective study design was employed. Pediatric patients admitted between August 2022 and August 2023 were reviewed. Out of 775 patient urine samples submitted for culture and sensitivity testing, 40 patients with a confirmed diagnosis of UTI were identified based on documented clinical symptoms, and laboratory findings. Descriptive statistics were utilized to analyze the prevalence rates of UTIs and the distribution of causative pathogens among the study population.

Result: 775 patients with complaints of UTI and associated risk factors were analyzed. Among these, 40 samples tested positive for UTI, while 735 had negative urine cultures. The overall prevalence rate of UTI was 5.2%. Of the 40 positive cases, 31 cases (77.5%) were attributed to gram-negative bacteria, while 9 cases (22.5%) were attributed to gram-positive bacteria. Among the gram-negative bacterial isolates, Escherichia coli was the predominant pathogen, accounting for 51.5% of cases, followed by Klebsiella at 12.5%.

Conclusion: This study enhances the understanding of the prevalence, epidemiological characteristics, and causative pathogens of pediatric UTIs in Tobruk, offering critical insights for improving diagnosis, treatment, and prevention strategies in pediatric healthcare. So that, healthcare providers can better manage UTIs in children, ultimately improving patient outcomes

KEYWORDS: Urinary tract infections; children; risk factors; Antibiotic Resistance.

INTRODUCTION

Urinary tract infections (UTIs) are common in pediatric populations and are among the most prevalent bacterial diseases worldwide, particularly in infants and young children, making them a significant pediatric health concern[1]. Among children, UTIs can manifest with varying degrees of severity and can be caused by a spectrum of pathogens. Understanding the specific pathogens responsible for these infections is crucial for effective diagnosis and treatment[2]. UTIs are particularly important to address because when they affect the upper urinary tract, they can form scars that damage the growing kidney and predispose individuals to hypertension and chronic kidney disease [3].

The incidence of UTIs among children has drawn significant attention due to its implications for pediatric healthcare management [4]**.** UTIs represent a frequent challenge, often necessitating hospitalization for proper diagnosis and management. These infections can arise from various causative pathogens, ranging from bacteria to fungi, each with its unique clinical implications and treatment considerations. Consequently, the prevalence of UTIs among children admitted to pediatric wards has become a focal point of research and clinical attention[5] [6] .

The American Academy of Pediatrics (AAP) clinical practice guidelines emphasize the importance of urine culture and the presence of pyuria for the diagnosis of UTI. Pyuria is identified by urinalysis showing more than or equal to 10 white blood cells (WBC)/mm³ or more than or equal to 5 WBC per high-powered field (HPF), or by the presence of leukocyte esterase (LE) on a dipstick. A positive urine culture is defined by the isolation of a single uropathogen at a density greater than 50,000 colony-forming units (CFU)/mL [5]**.**

The pathogenesis of UTIs involves the ascent of bacteria from the periurethral area, migrating in a retrograde fashion through the urethra to reach the bladder and potentially the upper urinary tract. Periurethral colonization with uropathogenic bacteria is a crucial factor in the development of UTIs. The increased susceptibility of girls to UTIs is attributed to the shorter female urethra and the frequent colonization of the perineum by enteric organisms. Hematogenous spread, although less common, can also occur, particularly in the first few months of life [7] [6] .

Many children with UTIs have structural abnormalities of the urinary tract [3] . The ultrasonography is recommended with the first-time febrile UTI to rule out structural abnormalities and detect hydronephrosis, which needs further evaluation such as voiding cystourethrography (VCUG). [8]

Understanding the epidemiology of UTIs and their causative pathogens is essential for effective diagnosis, treatment, and prevention strategies. This study aims to estimate the prevalence of UTIs, the epidemiological patterns, and the distribution of causative pathogens among children admitted to the pediatric department of Tobruk Medical Center over the past year. By analyzing these trends, we hope to provide insights that will enhance the management of UTIs in pediatric patients and improve outcomes.

PATIENTS AND METHOD

To estimate the prevalence of urinary tract infection (UTI) and to determine the causative pathogens among children admitted to the pediatric department at Tobruk Medical Center, a retrospective study design was employed. Medical records of pediatric patients admitted to the pediatric ward between August 2022 and August 2023 were reviewed, focusing on cases suspected of having UTIs. Infants and children under 15 years of age were included in the study population. Relevant demographic data, clinical presentations, and microbiological results were extracted from the patients' medical records.

Urinary tract infection was defined as bacterial growth of >10^5 CFU/mL in urine culture. Patients with a colony count meeting this criterion were considered to have a confirmed diagnosis of UTI.

Out of 775 patient urine samples submitted for culture and sensitivity testing, 40 patients with a confirmed diagnosis of UTI were identified based on documented clinical symptoms, laboratory findings, and pediatrician diagnoses. Descriptive statistics were utilized to analyze the prevalence rates of UTIs and the distribution of causative pathogens among the study population.

RESULTS

In this study, 775 urine samples from pediatric patients with complaints of UTI and associated risk factors were analyzed. Among these, 40 samples tested positive for UTI, while 735 had negative urine cultures. The overall prevalence rate of UTI was 5.2%.

Table (1): Distribution of the studied cases regarding the incidence of UTIs

CASES	Number	Percent
Positive	40	5.2
Negative	735	94.8
Total	775	100

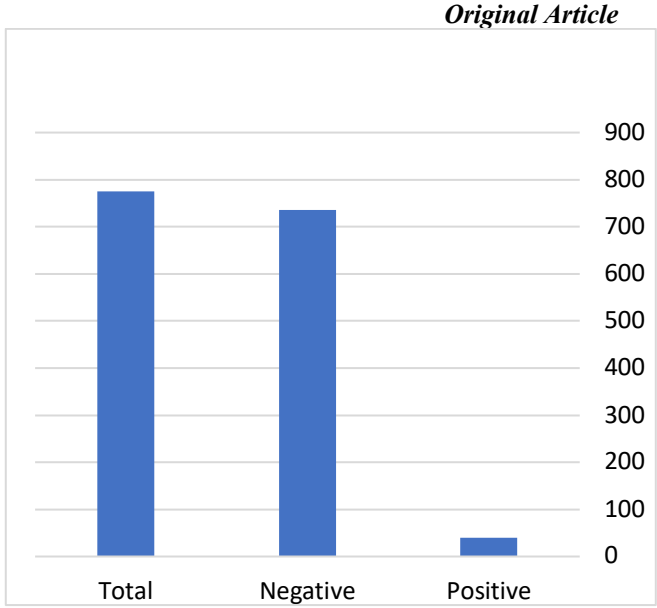

Fig. (1): Distribution of the studied cases regarding the incidence of UTIs

Table (2): shows the distribution of UTIs according to gender. Cases with positive urine culture in males were 16(40%), while in females were 24(60%).

Gender	no.	%
Male	16	40
Female	24	60
Total	40	100

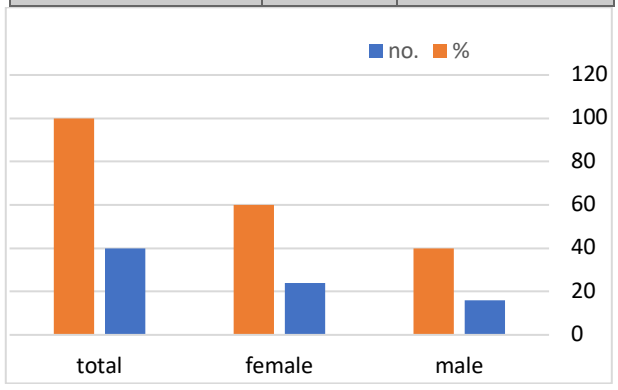

Fig. (2): Distribution of UTIs cases according to gender

The majority of positive cases were in the age group 0-1 year, with 22 cases comprising 55% of the total positive cases. Of these, 15 cases (68%) were male, while 7 cases (32%) were female. Above the age of one year, the majority of cases were female.

Table (3): Distribution of the positive cases according to age groups

Age group Years	Total no.	Male	Female
0 – 1	22	15	7
1 – 5	11	0	11
5 - 10	5	0	5
10 - 15	2	1	1

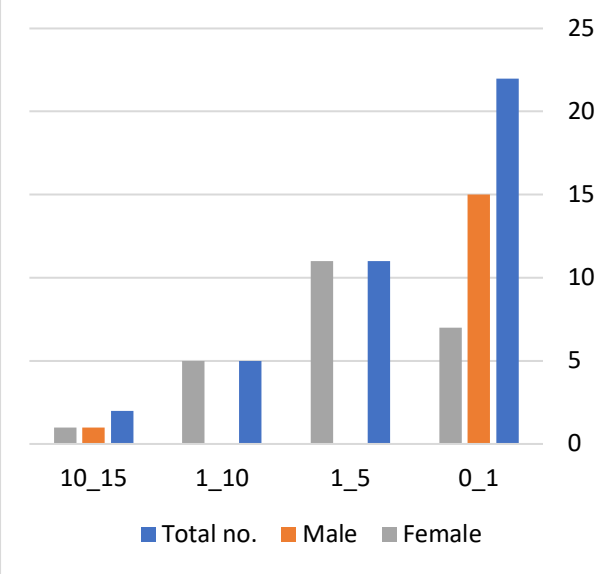

Fig (3): Distribution of the positive cases according to age groups

Regarding place of residence, 31 cases from urban areas, while 9 cases were from rural areas.

Table (4): Distribution of positive cases by place of residence.

Residence place	Positive	
	No.	%
Urban	31	77.5
Rural	9	22.5
Total	40	100.0

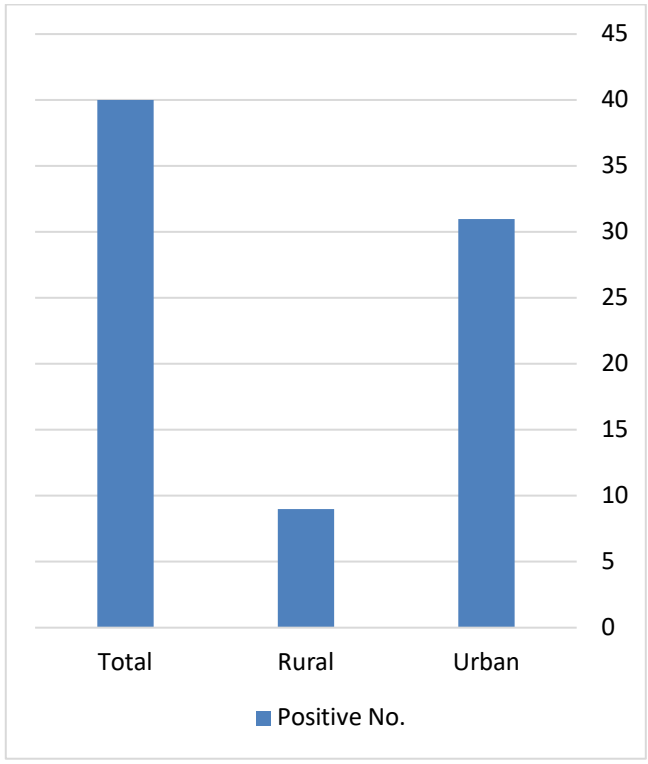

Fig (4): Distribution of positive cases according to place of residence.

Of the 40 positive cases, 31 cases (77.5%) were attributed to gram-negative bacteria, while 9 cases (22.5%) were attributed to gram-positive bacteria. Among the gram-negative bacterial isolates, Escherichia coli was the predominant pathogen, accounting for 51.5% of cases, followed by Klebsiella at 12.5% and Pseudomonas at 5%. Among the gram-positive bacteria, Staphylococcus was the predominant isolate, accounting for 12.5% of cases, followed by Enterococcus at 5%.

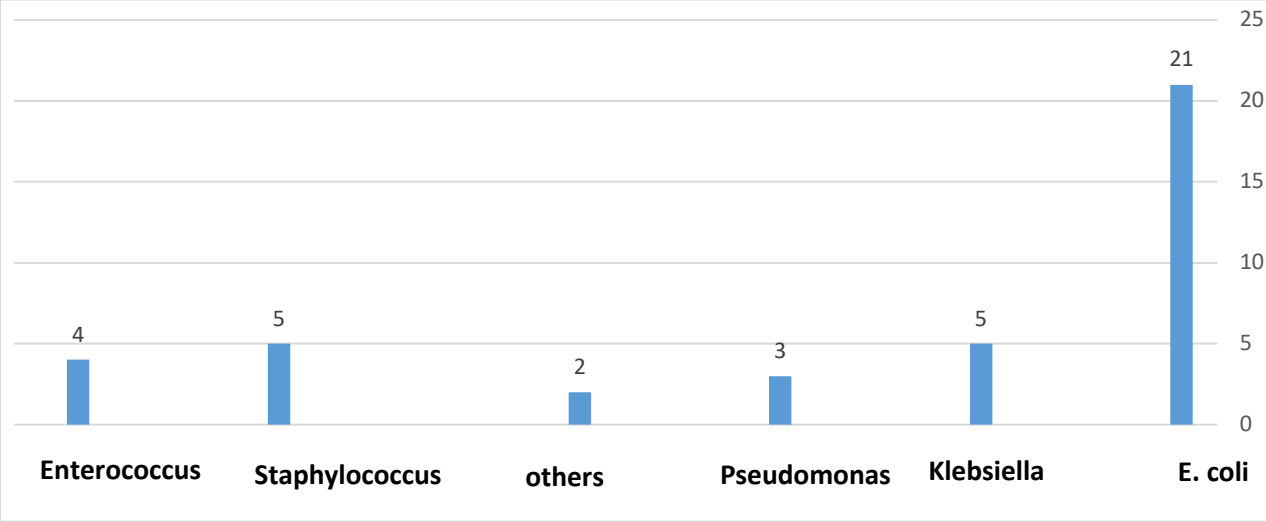

Fig. (5): Distribution of causative pathogens by positive cases

Gram stain				No.	%
Gram negative	31	77.5	**E. coli**	21	52.5
			Klebsiella	5	12.5
			Pseudomonas	3	37.5
			others	2	5
Gram positive	9	22	**Staphylococcus**	5	12.5
			Enterococcus	4	10

Table (5): Distribution of causative pathogens by positive cases

Regarding the ultrasound examination findings, 36 cases were normal, 3 cases had hydronephrosis, and one case had pelvis-ureteric junction stenosis (PUJ).

Table (6): Distribution of positive cases according to ultrasound examination finding.

Ultrasound examination finding	Positive	
	No.	%
Normal	36	90
Hydronephrosis	3	7.5
PUJ-Stenosis	1	2.5

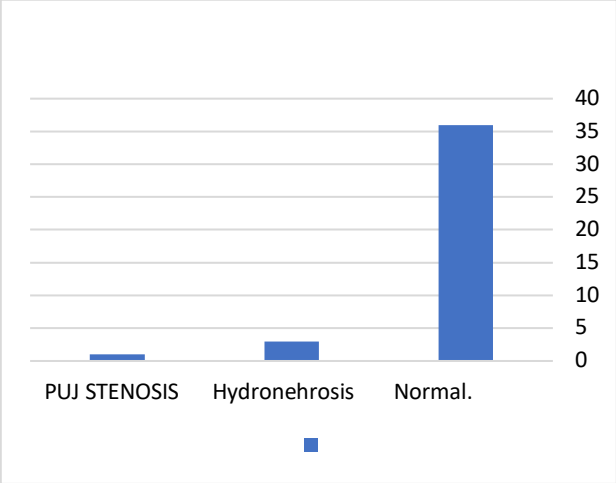

Fig (6): Distribution of positive cases according to ultrasound examination finding.

DISCUSSION

The results of this study provide a comprehensive overview of the prevalence, epidemiology, and causative pathogens of urinary tract infections (UTIs) among pediatric patients admitted to Tobruk Medical Center.

Prevalence and Demographic Distribution

The overall prevalence of UTIs in this pediatric population was found to be 5.2%. This prevalence aligns with other studies that have reported similar rates of UTIs in children, indicating that UTIs are a common issue in pediatric healthcare [4]. Notably, the study found that the majority of positive cases were in the age group of 0-1 year, with 55% of the total positive cases. Within this age group, males were disproportionately affected (68%). This gender distribution in infants could be attributed to factors such as uncircumcised status in male infants and congenital abnormalities that predispose them to UTIs, which contrasts with the general trend observed in older children where females are more commonly affected by UTIs [9] [10].

Above the age of one year, the majority of UTI cases were female, which is consistent with the established understanding that females have a higher risk of UTIs due to anatomical and physiological differences[5]. This shift in gender distribution underscores the importance of targeted prevention and intervention strategies based on age and gender.

Pathogen Distribution

The study identified a predominance of gram-negative bacteria (77.5%) in UTI cases, with Escherichia coli being the most common pathogen, accounting for 51.5% of cases. This finding conforms to reports from Sudanese children, where E. coli was the most commonly isolated pathogen (60%) [11] and also in Gulf region [12]. This finding is also in line with global

data indicating that E. coli is the leading cause of UTIs in both adults and children [6] [7]. The second most common organism was Klebsiella, seen in 12.5% of cases, similar to observations in Saudi Arabia [10]. The presence of other gram-negative bacteria such as Klebsiella (12.5%) and Pseudomonas (5%) highlights the diversity of pathogens that can cause UTIs and the need for broad-spectrum empirical antibiotic coverage [13]. Gram-positive bacteria were responsible for 22.5% of UTI cases, with Staphylococcus accounting for 12.5% and Enterococcus for 5% of the cases. The inclusion of gram-positive pathogens in the etiological profile of UTIs is essential for developing effective treatment protocols, particularly in cases where initial empirical therapy may not cover these organisms [6] [14].

Clinical Implications

The high prevalence of E. coli underscores the need for empiric antibiotic therapy that effectively targets this pathogen. However, the presence of other gram-negative and gram-positive bacteria necessitates ongoing surveillance of antimicrobial susceptibility patterns to ensure appropriate empirical therapy [15] . This is crucial for preventing the development of antibiotic resistance, which remains a significant concern in pediatric UTI management [7] [16] .

Moreover, the significant proportion of UTIs in infants and young children suggests a need for heightened vigilance and possibly more aggressive diagnostic approaches in this age group. Given the difficulty in distinguishing between cystitis and pyelonephritis based on clinical presentation alone, particularly in infants, prompt and accurate diagnosis is essential to prevent complications such as renal scarring [9] [2] .

Limitations and Future Directions

While this study provides valuable insights, it is not without limitations. The retrospective design may be subject to biases related to the completeness and accuracy of medical records. Additionally, the study is limited to a single medical center, which may affect the generalizability of the findings to other settings. Future studies could focus on prospective data collection and multi-center collaborations to validate these findings and explore regional variations in UTI pathogens and resistance patterns. Further research into the risk factors contributing to the high prevalence of UTIs in male infants could also provide more tailored preventive strategies

CONCLUSION

This study provides an investigation of the prevalence, epidemiological factors, and causative pathogens of urinary tract infections (UTIs) among children admitted to the pediatric department of Tobruk Medical Center. The overall prevalence rate of UTIs in this pediatric population was found to be 5.2%, highlighting the significance of this condition in childhood.

Infants, particularly males under the age of one year, were disproportionately affected, accounting for 55% of the total positive cases. This suggests that specific factors such as uncircumcised status and congenital abnormalities in male infants contribute to the increased susceptibility in this age group. Above the age of one year, females being more commonly affected, consistent with broader epidemiological patterns observed in other studies.

Pathogen distribution analysis revealed that gram-negative bacteria were the predominant causative agents, with Escherichia coli accounting for 51.5% of the cases, followed by Klebsiella and Pseudomonas. Gram-positive bacteria, primarily Staphylococcus and Enterococcus, were also significant contributors to the UTI cases. This pathogen profile underscores the need for empirical antibiotic therapy that effectively targets both gram-negative and gram-positive organisms to ensure comprehensive treatment.

In conclusion, this study enhances the understanding of the prevalence,

epidemiological characteristics, and causative pathogens of pediatric UTIs in Tobruk, offering critical insights for improving diagnosis, treatment, and prevention strategies in pediatric healthcare. So that, the healthcare providers can better manage UTIs in children, ultimately improving patient outcomes.

ACKNOWLEDGEMENT

Conflict of interest: The authors declare that they have no conflict of interest**.**

Funding: This research received no funding.

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Tobruk University Journal of Medical Sciences (TUJMS): ISSN No 2789-2093 Volume (8): Issue 1 July 2024
Original Article

Prevalence of Ischemic Heart Disease and the associated Risk Factors Among Libyan Patients in Tobruk City

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Website: https://medicine.tu.edu.ly/journal/4514/

Received: 04/06/2024/ Accepted: 10/06/2024

ABSTRACT:

Background: Acute coronary syndrome (ACS) is a common presenting problem with frequent diagnostic uncertainties.
Aims: to assess the prevalence of ischemic heart disease (IHD) and the associated risk factors among Libyan patients in Tobruk City.
Methods: A cross-sectional study was conducted on 40 Libyan patients admitted to the cardiac care unit in TobrukCity. Patients underwent a comprehensive evaluation, including history taking, physical examination, electrocardiography, and blood tests. In addition, data on traditional cardiovascular risk factors such as hypertension, diabetes mellitus, smoking, and obesity were recorded.
Results: The mean age of the 40 patients was 55.61 ± 9.08 years. Males constituted 62.5% (25/40) of the cases, and high-risk age (≥45 years for males and ≥55 years for females) was prevalent in 75.0% of the study population. Obesity was identified in 12.5% (5/40) cases, while overweight status was observed in 47.5% (19/40) cases. Overall, 35.0% (14/40) of the cases were diagnosed as hypertensive. Dyslipidemia was found in 15.0% (6/40) of patients, while diabetes mellitus was present in 27.5% (11/40) of the study population. Multivariate analysis revealed that high-risk age and hypertension were significant independent predictors of ischemic heart disease (IHD). The analysis also showed significant associations between diastolic and systolic blood pressure, smoking habits, and IHD.
Conclusion: This study found a high prevalence of traditional cardiovascular risk factors among patients with suspected acute coronary syndrome in Tobruk, Libya. Early identification and management of these risk factors are crucial for preventing IHD and its complications.

Keywords: Cardiovascular diseases, Acute coronary syndrome, smoking, ischemic heart disease, risk factors

INTRODUCTION

Ischemic Heart Disease is the first-ranked and the most common cause of death in Cardiovascular and overall disease. The estimated prevalence of IHD among people aged ≥18 years in 2013 was 6.1, 6.4, 5.3, and 3.7% in Caucasian, African, Latino, and Asian populations, respectively. The prevalence increased with age and more prevalence was noted among males and IHD increased in patients with potential risk factors such as diabetes mellitus (1). Coronary artery disease is the most common cause of death in cardiovascular disease. The rate of morbidity and mortality is high, and the costs incurred for the treatment process are also very high, thus having a bad impact on the welfare and quality of life in patients, families, and health costs borne by the state. The proper management can reduce the number of losses. (2) Stable coronary artery disease (SCAD) is generally characterized by episodes of reversible myocardial demand/supply mismatch, related to ischemia or hypoxia, which are usually inducible by exercise, emotion, or other stress and reproducible. However, it may also occur spontaneously. (3)

Coronary artery disease is most commonly caused by the inability of atherosclerotic coronary arteries to perfuse the heart due to partial or total occlusion of the coronary arteries. (4) Stable coronary artery disease also includes the stabilized, often asymptomatic, phases that follow an ACS. SCAD has various clinical presentations that are associated with different underlying mechanisms that mainly include: plaque-related obstruction of epicardial arteries, focal or diffuse spasm of normal or plaque-diseased arteries, microvascular dysfunction, and left ventricular dysfunction caused by prior acute myocardial necrosis and/or hibernation. (5) Ischemic means that an organ (e.g., the heart) is not getting enough blood and oxygen. Ischemic heart disease, also called coronary heart disease (CHD) or coronary artery disease, is the term given to heart problems caused by narrowed heart (coronary) arteries that supply blood to the heart muscle. Although the narrowing can be caused by a blood clot or constriction of the blood vessel, it is caused by a buildup of plaque, calledatherosclerosis. When the blood flow to the heart muscle is completely blocked, the heart muscle cells die, which is termed a heart attack or myocardial infarction (MI). (6)

PATIENTS AND METHODS

This cross-sectional study was done in the cardiac care unit of 40 Libyan patients in Tobruk City. Moreover, written informed consent was obtained from all patients, and the study was approved by the research ethical committee of the Faculty of Medicine, Tobruk University (Institutional Review Board).

Inclusion criteria:

Patients admitted either for:

1- Elective coronary angiography.
2- Coronary angiography with acute coronary syndrome.3- Available during the period of data collection.

Exclusion criteria:

1- Seriously ill.
2- Suffering from mental disorders.

Sampling technique and sample size determination:

The participants were chosen by systematic random sampling from the Libyan patients admitted to the cardiac care unit in Tobruk City.

All patients included in the study were subjected to the following:

History taking: including Personal history (name, age**,** and gender), Complaints of present illness: (onset, course, duration).

History of the present illness: including Cardiovascular risk factors such as

hypertension, diabetes mellitus,smoking, and prior myocardial infarction. History of addiction, symptoms suggestive of cardiac disease, History of drug intake: current medications. History of systemic disease. Family history of IHD.

A general examination: was performed, with the estimation of body weight and height which was obtained while the patient was putting on light clothes without shoes using a stadiometer and electronic scale in the CCU after confirming thepatient's stable condition. Body mass index was calculated as weight (kg)/ height (m2).

Vital signs: included heart rate (beats per minute) and blood pressure measured two separate times using a mercury sphygmomanometer with the cuff at the brachial artery, in both upper limbs, and averaged.

The smoking habit: was stratified according to the number of cigarettes smoked per day and the duration of smoking. Dietary data was collected through interviews regarding type of food (vegetarian or non-vegetarian).

For physical activity: the definition of the Indian consensus group was usedaccording to which a person is considered to have sedentary behavior if he walks less than 14.5 km a week. Body mass index (BMI) was computed as weight in kg/meter^2. Obesity was defined as a BMI of >27 kg/m^2 and overweight was defined as a BMI of >25 kg/m^2. Figures for criteria laid down by the Indian consensus group for being overweight (>23.5 kg/m^2) were also calculated **(8)**. Hypertension was diagnosed when the systolic blood pressure was 140 mmHg or more and the diastolic blood pressure was 90 mmHg or more, as per the guidelines of the British Hypertension Society **(9)**. Twelve lead electrocardiograms (ECGs) were taken using a BPL 108 ECG machine on each individual. Each ECG was reviewed by a cardiologist. A maximum of three visits were conducted for those individuals who could not be contacted during the first visit.

Criteria for the Diagnosis of IHD

The criteria for the diagnosis of IHD were: (a) a history of angina or infarction and previously diagnosed disease, (b) an affirmative response to the Rose questionnaire, and (c) electrocardiographic findings.

The presence of all three criteria was taken as confirmation of the diagnosisof coronary artery disease. The prevalence of coronary artery disease was also classified according to the presence or absence of symptoms. Those who knew they had the disease or showed an affirmative response to the Rose questionnaire were classified as symptomatic patients.

1. **Cardiac and chest examinations:** were done to detect any cause or sequelae of ACS (i.e. congestive heart failure, mitral regurgitation, or ventricular septal defect, etc.….).
2. **Diabetes mellitus was defined according to the American Diabetes Association(7):** Fasting plasma glucose ≥ 126 mg/dL, or 2-hour plasma glucose ≥200 mg/dL during standardized 75-g oral glucose tolerance test, or symptoms of hyperglycemia plus non-fasting plasma glucose ≥ 200 mg/dL, or HbA1c ≥ 6.5%.
3. **Obesity was defined as** BMI ≥ 30 Kg/m2 **(8)**
4. **Systemic hypertension was defined as** a usual office blood pressure of 140/90 mm Hg or higher **(9)**.

Data Collection, Processing, and Analysis:

Data were collected manually according to variables of the study mentioned above in a hard data sheet, then data entry was made according to aunified code. The source of data was the clinical data and measurements.

Statistical Analysis

The statistical package for social science (SPSS) software version 23.0 (IBM) has been used to

analyze data with the use of Chi-square (and Fisher exact test accordingly) as well as specificity, sensitivity, and predictive values were calculated considering angiography results (any positive result and multi-vessel disease) as a golden standard.

RESULTS

This cross-sectional study was carried out in the cardiac care unit on 40 Libyan patients admitted to the cardiac care unit in Tobruk City to assess the prevalence of ischemic heart disease (IHD) and the associated risk factors among Libyan patients in Tobruk City. All patients underwent diagnostic evaluation of acute coronary syndrome.

Table 1: Gender and High-risk age and ACS

High risk age	ACS		Total
	Acute cardiac condition	Other conditions	
Male	16	9	25
	40	22.5	62.5%
Female	6	9	15
	15.0%	22.5%	27.5%
High risk age	20	10	30
	50%	25%	75%
Low risk age	2	8	10
	5%	20%	25%
Total	55	45	100

Fisher's Exact Test P = 0.025 (Significant)

The study analyzed data from 40 patients with acute coronary syndrome (ACS). The mean age of the patients was 55.61 ± 9.08 years. Males constituted 62.5% (25/40) of the cases, and high-risk age (≥45 years for males and ≥55 years for females) was prevalent in 75.0% of the study population.

Table 2: Obesity, Overweight, Hypertensive, High Systolic Pressure, High diastolic Pressure and ACS

High risk age	*ACS*		*Total*
	Acute cardiac condition	Other conditions	
Obese	2	3	5
	5.0%	7.5.0%	12.5%
Non	20	15	35
	50%	37.5%	87.5%
Overweight	11	8	19
	27.5%	20%	47.5%
Non	11	10	21
	27.5%	25%	52.5%
Hypertensive	11	3	14
	27.5%	7.5%	35%
None	11	15	26
	27.5%	37.5%	65%
High Systolic Pressure	9	3	12
	22.5%	7.5%	30%
Normal range	13	15	28
	32.5%	37.5%	70%
High diastolic Pressure	7	2	9
	17.5%	5%	22.5%
Normal range	15	16	31
	37.5%	40%	77.5%

Obesity was identified in 12.5% (5/40) of cases, while overweight status was observed in 47.5% (19/40) of cases. Hypertension was notable, with high systolic blood pressure recorded in 30.0% (12/40) of cases and high diastolic blood pressure in 22.5% (9/40) of cases. Overall, 35.0% (14/40) of the cases were diagnosed as hypertensive (Table 2).

Table 3: Dyslipidemia, Diabetic, CKD Stage ≥ III, CRP and ACS (n = 40)

Dyslipidemia	ACS		Total
	Acute cardiac condition	Other conditions	
Dyslipidemia	4	2	6
	10%	5%	15%
Non	18	16	34
	45%	40.1%	85%
Diabetic	3	8	11
	7.5%	20%	27.5%
Non	19	10	29
	47.5%	25%	72.5 %
CKD (eGFR <60)	2	6	8
	5%	15%	20%
CKD (eGFR ≥60)	20	12	32
	50%	30%	80%
CRP Positive	2	6	8
	5%	15%	20.0%
CRP Negative	20	12	32
	50%	30%	80%

Dyslipidemia was found in 15.0% (6/40) of patients, while diabetes mellitus was present in 27.5% (11/40) of the study population. Chronic kidney disease (CKD) stage III or higher was noted in 20.0% (8/40) of cases (Table 3).

Table 4: Multivariate analysis for factors affecting ACS

Factor	B	Wald X^2	P	OR	95% C.I. for OR	
Risky age	2.203	3.820	0.049	9.055	0.994	82.502
Gender	0.126	0.010	0.920	1.134	0.098	13.092
High Systolic Pressure	-0.481	0.000	1.000	0.618	0.000	.
High Diastolic Pressure	-20.087	0.000	0.999	0.000	0.000	.
Hypertension	40.086	0.000	0.999	> 1000.0	0.000	.
Diabetic	-0.385	0.104	0.747	0.680	0.065	7.079
CKD Stage ≥III	-1.105	0.426	0.514	0.331	0.012	9.140
Thromboembolic	-39.644	0.000	0.998	0.000	0.000	.
CRP	-1.060	0.770	0.380	0.347	0.033	3.696
Constant	21.473	0.000	0.999	>1000.0		

B Beta coefficient of regression, OR odds ratio, CI Confidence interval, CKD chronic kidney disease, CRP C reactive protein. Multivariate analysis revealed that high-risk age and hypertension were significant independent predictors of ischemic heart disease (IHD). The study also showed significant associations

between diastolic and systolic blood pressure, smoking habits, and IHD (Table 4).

DISCUSSION

Cardiovascular disease (CVD) is a group of diseases that include both the heart and blood vessels, thereby including coronary heart disease (CHD) coronary artery disease (CAD), and acute coronary syndrome (ACS). During the last decades, the coronary artery disease mortality rate has significantly decreased in developed countries, the fact that could be attributed to both primary and secondary care and prevention programs. Despite the continued efforts to control cardiovascular risk factors, IHD remains the leading cause of death worldwide, in both developed and developing countries. [10]

Ischemic heart disease is considered a multifactorial disease with a complex pathophysiology generated by the combined effects of genes and the environment. Environmental influences have been widely investigated, but genetic markers have not been fully understood. [11]

Acute coronary syndrome (ACS) is the acute manifestation of IHD that results from the formation of a platelet-rich thrombus over an atherosclerotic plaque within a coronary artery and/or a plaque rupture. The symptoms and severity of ACSs (unstable angina [UA] and myocardial infarction) vary depending on the degree to which thrombi occlude the coronary arteries. [12]

Although IHD occurs usually in patients older than 45 years, younger patients having IHD have also been reported. Premature IHD is a growing entity that carries significant morbidity, psychological effects, and financial constraints for both patients and their families. In young people, the role of genetic risk factors is expected to be even more important than that of environmental factors. [13] This cross-sectional study was carried out cardiac care unit among the Libyan patients in Tobruk city on 50 patients admitted in cardiac care unit among the Libyan patients in Tobruk city to assess the prevalence of IHD and the associated risk factors among the Libyan patients in Tobruk city.

The study analyzed data from 40 patients with acute coronary syndrome (ACS). The mean age of the patients was 55.61 ± 9.08 years. Males constituted 62.5% (25/40) of the cases, and high-risk age (≥45 years for males and ≥55 years for females) was prevalent in 75.0% of the study population. Obesity was identified in 12.5% (5/40) cases, while overweight status was observed in 47.5% (19/40) cases. Hypertension was notable, with high systolic blood pressure recorded in 30.0% (12/40) cases and high diastolic blood pressure in 22.5% (9/40) cases. Overall, 35.0% (14/40) of the cases were diagnosed as hypertensive.

Dyslipidemia was found in 15.0% (6/40) of patients, while diabetes mellitus was present in 27.5% (11/40) of the study population. Chronic kidney disease (CKD) stage III or higher was noted in 20.0% (8/40) of cases.

Multivariate analysis revealed that high-risk age and hypertension were significant independent predictors of ischemic heart disease (IHD). The analysis also showed significant associations between diastolic and systolic blood pressure, smoking habits, and IHD.

Similar to our study, **Zhao et al.,** [14] reported that the age of the patients ranged from 19–90 years old with a mean age of 54 years, 77.6% of the patients were males. Also, this agrees with a study conducted by **Langsted et al.,** [15] who reported that a total of 468 participants responded. Male participants comprised 41.2% of the recruited participants (n=193) (20.73 ± 3.32) years and (23.80 ± 7.37) years.

The present study showed that the prevalence of IHD was 6 (12 %), A Lower prevalence was reported by **Al Rawahi et al.,** [16] who studied

cardiovascular disease incidence and risk factor patterns among Omanis the overall cumulative incidence of CVD was 9.4% with an incidence density of 17.6 per 1000 person-years.

In the present study of cardiovascular risk factors, Binary logistic regression analysis shows that 90 % of IHD can be explained by predictor variables. Diastolic blood pressure, systolic blood pressure, and smoking habits were significantly associated with IHD, whereas no significant relation was found between age, sex, physical activity, body mass index, or diet habits. On the other hand, a study conducted by **Al Rawahi et al., [16]** reported a high prevalence of most of the traditional risk factors such as obesity, poor glycemic control, HTN, dyslipidemia, and albuminuria.

Some of the traditional factors have been excluded in the univariate association with CVD risk. For example, current smoking in the present study showed a low prevalence (74%) and was associated with CVD. A longitudinal study among English patients showed the prevalence of current smoking to be around 34% in men and 25% in women, while in New Zealand it was 15%. [17]

Other global studies even with low CVD incidence have also shown a higher prevalence of smoking among the study groups. [18] Cigarette smoking played a critical role in the development of premature CHD, reducing the age at trial entry by about 1 decade in every risk factor subgroup. In addition, more than 70% of the 12154 patients aged 45 years or younger were current cigarette smokers. Although cigarette smoking is harmful at any age, the increase in relative risk of coronary events in young persons is particularly magnified given their baseline low risk. Furthermore, cigarette smoking acts synergistically with other conventional risk factors, greatly increasing the baseline risk associated with each risk factor individually. Thus, the elimination of cigarette smoking is of dramatic public health importance because it could delay the onset of CHD by a decade [19].

The prevalence of smoking among Libyan diabetic patients was high; this calls for incorporating smoking cessation services within the diabetes careclinics. Has been observed by many studies The overall prevalence of current smoking was 26.4% and past smoking 40.3% [20]. This may be explained by the social and cultural factors towards this habit, these are the potential reasons for the significant association between smoking and CVD observed in this study.

The explanations of results in the present study are centered around that the study population is already a part of a high-risk population that presented for different causes of chest pain and might not diagnosed as ACS for technical reasons. Anyhow, stable angina may be underdiagnosed as it was notan outcome in the present study.

CONCLUSION

Patients presenting with chest pain generally have a high rate of traditional cardiac risk factors. There were significant associations detected between ACS with high-risk age, hypertension, and smoking were significantly associated with IHD. Considering risk factors in handling and diagnosis of cases with chest pain is important. Screening and treatment for risk factors may be worthwhile in preventing cardiac disease.

RECOMMENDATIONS

Considering risk factors in handling and diagnosis of cases with chest pain is important. Screening and treatment for risk factors may be worthwhile in preventing cardiac disease.

ACKNOWLEDGEMENT

No Conflict of Interest

No financial disclosure

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Benefits and Risks of Green Tea in Libyan Meals and the Safe Method of Preparing it and Nutritive Advices

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Received: 23/05/2024 Accepted: 07/06/2024

ABSTRACT:

This effort clarifies the percentage of daily tannin and caffeine in Libyan green tea beverages. Also, it is to know the extent of Libyan's awareness of the healthy method of preparing green tea. As well as it is to know the nutritional and healthy benefits of green tea and nutritive advice. 12 samples of *vert de chine* of green tea were analyzed. 12 samples were divided into four groups. Each group contained three samples. The preparations include raw samples steeped in boiled water, samples heated for 5 minutes, samples heated for half an hour, and samples heated for an hour. The heating temperature was at 100C˚. A cross-sectional study was randomly achieved on 200 healthy Libyan people (100 men and 100 women) aged from 30 to 45 years old from 1 January to 31 March 2023 in Derna, Libya. The questionnaire was about the type of preparation method of green tea, boiling time, and their knowledge about the nutritional benefits and health risks of green tea beverages. The caffeine percentages were 1.6 %, 2.1 %, 2.5%, and 4.7%, respectively. Also, the highest tannin participation in green tea (heated for an hour) was 91 mg/100ml. This value is higher than the values in raw green tea steeped in boiled water and heated for 5 minutes, which were 0.109 mg/100 ml and 0.49 mg/100 ml, respectively. The heating time affects the proportion of caffeine and tannin in green tea drinks; as the heating time rises, the percentage of caffeine and tannin rises**.** There was a statistically significant difference between percentages of caffeine and tannin according to their heating times (P= 0.031). The proportion of tannin in green tea was higher than caffeine. The expansion of tannin percentage in green tea may lead to genetic faults and other problems. This indicates that raw green tea steeped in boiled water and boiled green tea for 5 minutes may nutritionally be regarded as the most excellent for improving human health because they provide the smallest percentages of caffeine and tannin in Libyan routinely prepared green tea drinks. However, no participant preferred to steep green tea in boiled water.

Key Words: Tannins, Caffeine, Libyan Green Tea, Healthy Method, Nutritive advices**.**

INTRODUCTION

Green tea is nutritionally described by nutritionists for supporting patients with clogged arteries, heart disease, osteoporosis, liver diseases, Parkinson's disease, high cholesterol, hypertension, diabetes, obesity, asthma, flu, and cold. Similarly, green tea supports people diagnosed with cancers including bladder, esophagus, pancreas, breast, colon, stomach, leukemia, mouth, and ovarian. prostate, and lung. (Wolfram et al, 2006; Pasrij and Anandhamakrishnan 2015). Green tea is one of the major expendable beverages in the earth. It is largely safe, healthy, and used as a food supplement because it contains a valuable number of polyphenols. Whereas, the other types of tea contain smaller amounts of polyphenols. These polyphenols in green tea include caffeine and tannin. Tannins are considered one of the acids found in tea that work as antioxidants. Caffeine keeps from heart diseases, cholesterol, hypertension, diabetes mellitus, cancer, and iron absorption and body control.

Nutritionally, green tea has medicinal and nutritional properties. Newly, it has a big deal of care because tea polyphenols are significant antioxidants (Devine et al. 2007; Farhoosh et al 2007; Florian et al. 2004; Hirasawa and Takada 2004, Jeu-Ming et al. 2015). Temperature and water steeping affects the antioxidant action and caffeine content in green tea. However, some phenolic compounds in green tea are not heat resistant and volatilized at high temperatures. Also, green tea reduces the absorption of non-heme iron; therefore, people should eat ascorbic acid-containing foods such as broccoli to enhance non-heme iron absorption (Tadelech Atomssa, 2011, Bhagwat et al 2021). High-tannin-containing tea may lead to a disturbance in the excess production of iron at rates that are harmful to human health. This disorder stems from a genetic defect of uncontrolled absorption of iron. While, a high amount of caffeine may lead to headaches, anxiety, irritability, and insomnia (Bhagwat et al. 2021). So, studying tannin and caffeine contents in routinely prepared Libyan green tea drinks should be highly considered merit. The present study aimed to determine the percentage of tannin and caffeine in routinely prepared Libyan green tea. Also, it is to know the extent of Libyan's consciousness of the healthy method to prepare green tea and its standing for health. As well as it is to identify the nutritional and healthy benefits and risks of green tea and nutritive advice.

MATERIALS AND METHODS

Chemical Manual Method

Four chemical manual methods for caffeine and tannin separation from green tea were performed. The chemical methods were applied based on Amal et al., 2021. Green tea samples were sold from Al-Tamzeny Shop, Derna, Libya. The devices used in this research were analytical balance, hot water, 1000 volume flasks, cooler, separating funnels, filter papers, crucible, and drier. The chemical solutions were ammonia solution, chloroform, Quinine sulfate, and 1N H_2SO_4. 12 samples of Libyan green tea *vert de chine chunmee* were divided into 4 groups and analyzed. Firstly, 3 samples were raw green tea steeped in boiled water, and secondly, 3 samples were heated for 5 minutes. Thirdly, 3 samples were heated for half an hour and fourthly, 3 samples were heated for an hour. The heating temperature was 100 ˚C. All samples for each group were prepared at a constant time. The separation of caffeine from green tea was manual. Approximately, 5 grams of green tea was added to 400 ml hot water in a 1000-volume flask with 5 ml craz I and 5 ml craz II, then it was completed to volume by hot water, then cooled and put in a separating funnel. About 10 ml of ammonia solution was added and separated by 25 ml chloroform 5 times and collected in the crucible. For manual separation of tannin, about 5 grams of green tea was added to 400 ml water, heated for 1 hour, and filtrated. 1 gram of quinine sulfate was added to 25 ml water, then 2.5 ml 1N H_2SO_4 was added. The final solution was filtrated. The precipitate was formed and dried in known weight.

Dietary Questionnaire Method

A cross-sectional study was randomly conducted on 200 Libyan people (100 men and 100 women) from Derna, Libya. The participants were in good health and aged from 30 to 45 years old. Derna is a city located in Eastern Libya. The questionnaire study was performed from 1 January to 31 March 2023. The participants were randomly selected from different places in Derna City including Al-Gebela, Al-Sahal, Sheha, Wadi Alnaga, Al-Dahar Alahmar, and Sayada Kadija. The questionnaire was about drinking green tea daily, the type of method for preparing green tea beverages, the time of boiling temperature, and their knowledge about the nutritional benefits and health risks of green tea.

Statistical Analysis

Descriptive statistics were performed using the SPSS Statistics Software Program (version 24,

Inc., Chicago, Illinois, USA). An independent T-test was achieved to evaluate the significance of the association between percentages of caffeine and tannin according to the temperature time of the separation method. In all tests, $\alpha < 0.05$ was regarded as statistically significant with a level of confidence intervals of 95% for statistical significance. Graphs of percentages of caffeine and tannins were built using Microsoft Office Excel 2021 program. The percentage formulas were calculated based on the Libyan National Center for Standards in the Nutrition Laboratory, Al-Arab Medical University, Benghazi, Libya.

RESULTS AND DISCUSSION

Chemical Analysis

In the present chemical analysis, there was a significant difference between percentages of caffeine in the four Libyan routine preparation methods of green tea. The average percentages of caffeine were 1.6 %, 2.1 %, 2.5%, and 4.7%, respectively (Table 1). In contrast, there also was a significant difference between percentages of tannins in the four routine preparations of Libyan green tea. The average percentages were 10.9 %, 49 %, and 83 %, 91 %, respectively. This indicates that caffeine and tannin contents increase with heat, as the percentage increases by increasing the heating time (Table 1). According to the Libyan National Center for Standards, the Libyan standard criteria ratio for caffeine in raw green tea steeped in boiled water was 1.5 %. While, the Libyan standard criteria ratio for tannin in raw green tea was 10.5 % (Amal et al., 2021). In the present work, the ratio of caffeine in raw green tea steeped in boiled water was 1.6%, whereas the ratio of tannin in raw green tea was 10.9 %.

Tannin %	Caffeine %	Preparation Method
10.9	1.6	**Raw (steeped in boiled water)**
49	2.1	**Heated for 5 minutes**
83	2.5	**Heated for half an hour**
91	4.7	**Heated for hour**

Table 1: Average Percentages of Caffeine and Tannin in Green Tea Samples

This chemical analysis implied that the way of green tea preparation may affect the contents of caffeine and tannin in green tea. One finding in this work is that the rise in the temperature during the preparation of green tea drinks will promote the participation of tannin. This indicates that a green tea sample boiled for an hour produces 0.91 mg/100 ml of tannin. This value is higher than the amount of tannin in raw green tea steeped in boiled water samples and the samples boiled for 5 minutes. They were

0.109 mg/100 ml and 0.49 mg/100 ml, respectively. In comparison, boiling the leaves of green tea for an hour led to yielding the highest amount of caffeine (0.047 mg/100 ml). Whereas, boiling the leaves of green tea for five minutes lead to produce the lowest content of caffeine (0.021 mg/100 ml). The raw leaves of green tea steeped in boiled water produced 0.016 mg/100 of caffeine.

The data demonstrated that there was a statistically significant difference between percentages of caffeine and tannin according to their heating times (P= 0.031). This implies that tannin and caffeine percentages increase with heat and time. Likewise, tannin percentages in the boiled green tea samples were higher than the caffeine percentages. Tannin may lead to genetic imperfection, irregular absorption of iron, and other health problems (Bhagwat et al. 2021). Therefore, the boiling time for preparing green tea beverages should be short to have a minimum amount of tannin. Thus, we can reduce the health risks of green tea. This points to that the preparation ways include steeping green tea in boiled water and boiling green tea for 5 minutes are nutritionally safe and the best for human health because they provide safe and healthy amounts of caffeine and tannin in green tea beverages.

Nutritional Questionnaire Study

The random questionnaire study was accomplished on 200 healthy young men and women from Derna, Libya. All participants confirmed that they drink one to three cups of green tea daily. Moreover, all participants know that green tea has many health advantages. All participants believe that green tea provides them with health and increases their ability to concentrate. They all prefer drinking it over all other types of tea or drinks. A previous study reported that green tea is the most common beverage on the earth and promotes good health (Devine et al. 2007). 5 % (10) of participants boil green tea for 5 to 10 minutes, while 45% (90) of participants prefer to boil green tea for half an hour. About 50% (100) of participants like to drink green tea after boiling it for an hour as shown in Figure 1.

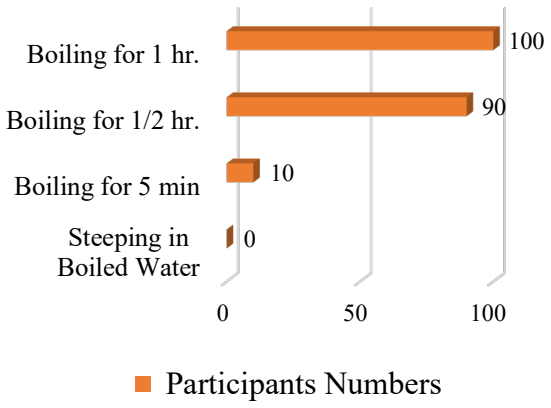

Figure 1. Preparations Ways for Libyan Green Tea.

Results exhibited that 25% (50) of participants knew that boiling green tea for a long time poses health risks. While 70% (140) of participants did not know that boiling green tea for an hour or more produces high amounts of tannin that enhance health damage. About, 5% (10) of participants knew that boiling green tea for a long time may harm health, but they did not care. Because they are accustomed to drinking this preparation way. Despite this, nutritionists select green tea as part of meal planning to improve many health problems that people suffer from, such as obesity, liver diseases, cancers, and others (Anandhamakrishnan 2015). Further research is needed to study people's awareness about the correct use of green tea drinks during daily life in a way that promotes human health.

NUTRITIONAL RECOMMENDATIONS

For good health, green tea strongly can improve alertness; however, continuous consumption of large amounts of caffeine in green tea may cause negative side effects such as headaches anxiety, and insomnia. Also, the increase in tannin levels in green tea drinks may cause genetic difficulties, abnormal iron concentrations, and other health risks. Since green tea is considered one of the most popular drinks in Libya, people should follow the following nutritional instructions to benefit healthily from drinking green tea (Newman, 2021; Jenna, 2008; Sinija and Mishra, 2008; Katiyar et al, 2007; Berube-Parent et al, 2005; Rietveld and Wiseman, 2003):

1-Visit a nutritionist to determine the appropriate amount of green tea drink according to health condition.

2- Drink good quality green boiled for five minutes without sugar.

3- Avoid steeping green tea for a long time.

4- Avoid drinking green tea on an empty stomach.

5- Avoid adding honey to very hot green tea beverages and do not drink tea before bed.

6- Add drops of lemon because vitamin C in lemon stimulates iron secretion.

CONCLUSION

Green tea contains tannin and caffeine. Tannins are considered one of the acids found in tea that work as antioxidants. Caffeine protects from heart diseases, cholesterol, hypertension, diabetes mellitus, cancer, and iron absorption and body control. Green tea beverage is beneficial for human health and protects it from many diseases that are harmful to the body. However, the method of preparing the tea affects the extent of its benefit or harm to the body. The tannin and caffeine

percentages increase with heat and time. The green tea samples prepared by boiling at 100˚C for an hour gave the highest percentage of caffeine (4.7 %) and the highest percentage of tannin (91 %) than other Libyan routine ways for preparing green tea. The rise of tannin percentage in green tea may lead to genetic problems, abnormal iron levels, and other health problems. Therefore, the boiling time during the preparation of green tea should be short. The study concludes that steeping green tea in boiled water and boiling green tea for 5 minutes may be nutritionally considered the best for improving human health. Both provide a smaller percentage of caffeine and tannin in green tea beverages.

Competing Interests

We (authors) declare that we have no conflict of interest.

ACKNOWLEDGEMENT

We acknowledge everyone who assists and supports this scientific work.

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