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Original Research Article

Estimation of Telomerase Enzyme Levels among Drug Users in Kirkuk City: A Cross-Sectional Study

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Abstract: ***Background***: Drug abuse is a chronic brain disorder affecting the dopaminergic system, characterized by a recurring cycle of intoxication and addiction. This study aimed to evaluate telomerase enzyme levels in drug users and their relationship to various demographic and health factors .***Methodology***: A cross-sectional study was conducted at the Drug and Psychotropic Substance Addiction Treatment Center in Kirkuk (Ministry of Interior). The study included 25 drug users of varying ages and backgrounds, all with pre-existing chronic conditions (diabetes, hypertension, and cancer), as well as 10 healthy individuals as a control group. .Blood samples were collected in yellow top blood tubes, centrifuged, and stored at -20°C. Telomerase enzyme levels were measured using ELISA .***Results*** **:**1- Socio-Demographic Characteristics .Type of Drug Used:- Our study results indicated that the majority of the sample (92%) used crystal meth, while the remaining percentages were distributed between marijuana and Lyrica to a lesser extent. Here, we note that the type of drug was not statistically significant in the distribution ($p = 0.412$). ***Marital and Method***: Half of the sample (48%) was married, followed by single individuals (32%). Regarding place of residence, 80% of the participants lived in the city center, with limited geographical distribution in other governorates such as Baghdad, Duhok, Erbil, and Sulaymaniyah .2- Associated Diseases of Addicts: The study revealed that the largest percentage of drug users suffered from hypertension (80%) among the sample, followed by diabetes. Statistically significant correlations ($p = 0.032$) were found regarding the distribution of these diseases among the sample, indicating that hypertension was the predominant characteristic in this group .Telomerase Enzyme Levels: When measuring telomerase enzyme levels, it was observed that diabetic patients had the highest average telomerase level (0.056531), while those with hypertension had an average level of 0.055248. The average enzyme level in healthy individuals was 0.054555 .***Conclusion*****:** Our study demonstrates a significant correlation between crystal meth use and hypertension in most participants. Telomerase enzyme levels showed slight variations based on the type of pre-existing condition, with those with diabetes and hypertension exhibiting higher levels compared to healthy individuals in the study.

Keywords: Crystal Methamphetamine, Telomerase Enzyme Levels, Hypertension, Chronic Diseases.

INTRODUCTION

Drug abuse causes serious disorders, leading to intellectual, social, and professional impairment due to the excessive consumption of substances such as alcohol, nicotine, and various drugs, including cannabis, sedatives, hypnotics, anti-anxiety medications, inhalants, opioids, hallucinogens, stimulants, and others. Drug abuse is classified based on its effects on the central nervous system. These effects range from increased energy and euphoria with stimulants like cocaine and methamphetamine to deep sedation resulting from sedatives such as heroin and fentanyl. These drugs affect dopamine levels in the midbrain, which is essential for their pleasurable effects. They produce their rewarding and addictive effects through direct activation of dopamine above the physiological level and indirectly by modulating other neurotransmitters (opioids, cannabis, glutamate, GABA, acetylcholine, and serotonin) in the brain's reward circuits (Ciucă Anghel *et al*., 2023).

Drug abuse involves a disruption in the regulation of the brain's motivational and reward pathways. The mesolimbic dopamine system, which includes the Nucleus Accumbens (NAc) and Ventral Tegmental Area (VTA), is involved in the release and regulation of dopamine, a neurotransmitter associated with pleasure and reinforcement. With drug abuse, this system becomes dysfunctional, leading to a hyperactive response to drugs and decreased sensitivity to natural rewards (Volko *et al*., 2019).

Drug use may lead to increased levels of protein kinase A (PKA) and cAMP (camp cyclic adenosine), and PKA-dependent phosphorylation in the nucleus accumbens. The withdrawal/negative phase involves an increase in corticotropin-releasing factor, norepinephrine, dynorphine, orexin, and substance P, and a concurrent decrease in serotonin, dopamine, opioid receptors, neuropeptide Y, pain receptors, oxytocin, and endocannabinoids. This decrease occurs in neural pathways involving the ventral tegmental area, the central nucleus of the amygdala, the bed of the peripheral nucleus, the cortex of the nucleus accumbens, and the chordae tendineae. These changes in neural circuits resulting from drug withdrawal lead to stressful psychological states including irritability, mood disturbances, pain, distress, depression, and loss of motivation for normal rewards. Human telomeres are repetitive protein structures of non-coding DNA, consisting of nucleotide sequences of consecutive TTAGGG repeats at the telomere ends. These chromosomes, along with their associated protective proteins, are essential for maintaining genome stability. Telomere length is the product of a balance between increased cell renewal and aging. When telomeres become too short, cell proliferation decreases, leading to cellular senescence (Navarro-Mateo *et al*., 2019). This is why telomere length has been considered an indicator of cellular aging. Over the past decade, shortened telomeres have been associated with increased incidence and mortality in several diseases, including chronic illnesses and drug abuse (Gruber *et al*., 2021).

Drug users have been found to have significantly shorter telomere lengths than the general population, with an average telomere length (0.061 telomeres/second) shorter than the average. This shortening is equivalent to approximately 38 years of average age-related telomere loss, the incidence of type 2 diabetes is inversely related to telomere length, and type 2 diabetes is equivalent to approximately 5 years of average age-related telomere loss. Age-related telomere shortening generally results from disruptions during cell division, oxidative stress, impaired antioxidant function, or interference with telomerase activity. Increased oxidative stress due to drug use is the most common mechanism of telomere shortening. Exposure to heroin, amphetamines, cocaine, and marijuana leads to a marked increase in oxidants, such as reactive oxygen species (ROS) and lipid peroxides, and a decrease in antioxidants, such as vitamin C and beta-carotene. The balance between oxidation and antioxidants is severely disrupted in drug users (Yang *et al.,* 2013).

The cumulative oxidative stress resulting from drug abuse causes oxidative damage to telomere DNA, as well as weakening antioxidant defense mechanisms and the elimination of free radicals, which may accelerate this damage and increase the rate of telomere shortening with each cell division, thereby reducing the expression of telomerase reverse transcriptase, an enzyme that plays an important and crucial role in telomerase activity and is regulated by redox-sensitive transcription factors, oxidative stress is not only associated with telomere shortening but is also associated with drug withdrawal symptoms. Determining whether telomere shortening represents a possible relationship to drug withdrawal symptoms requires further studies (Barnes *et al*., 2019).

MATERIAL AND METHODS

1- Experimental Design

A cross-sectional study was conducted on 25 drug addicts. Participants were divided based on several criteria (type of drug, presence or absence of chronic diseases, and marital status).

2- Data Collection: A questionnaire was used to collect basic information from the participants, including:

- **Type of drug:** crystal meth, marijuana, and Lyrica pills.
- **Medical History**: Participants were divided according to their chronic diseases (diabetes, hypertension, and cancer).
- **Geographical Distribution**: Participants were located in the following areas: Kirkuk, Baghdad, Duhok, Kut, Erbil, and Sulaymaniyah.

3- Laboratory Analyses: To measure telomerase enzyme levels, the following was performed:

- Blood samples were collected under sterile conditions using blood collection tubes.
- Telomerase enzyme concentration was measured using ELISA, and the results were obtained with high accuracy to within five decimal places (0.055248).

4- Statistical Analysis

SPSS version 25 was used, and the analysis was performed according to the statistical description (frequency extraction and percentages) to describe the qualitative variables. The arithmetic mean, minimum, and maximum telomerase

levels were calculated for each disease category. Additionally, ANOVA and Chi-Square tests were used to assess the probability level (P-value), with a statistical significance of $P < 0.05$.

RESULT

The results of our study, shown in Fig 1 (distribution of participants by drug type), indicate that the differences at the 0.05 significance level do not reach statistical significance. This may be due to the small sample size of 20, which could affect the detection of significant differences, or to a strong bias in the distribution towards one side. The Fig shows that crystal meth had the highest prevalence rate by a significant margin, with 23 users, representing 92%, compared to marijuana and Lyrica users, who had low or equal rates of 4% each.

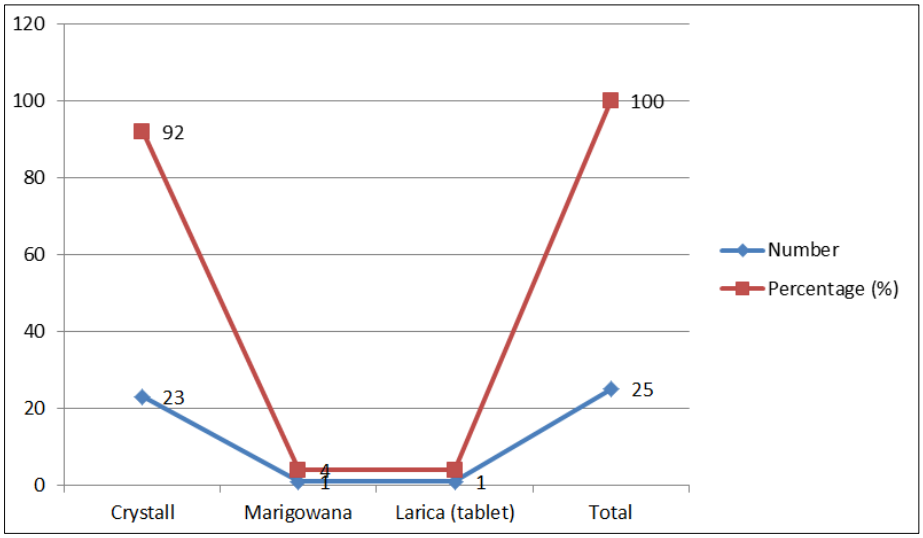

Fig. 1: Distribution of Study Subjects by Type of Drug P= 0.412 (NS)

Fig 2 (Distribution of Participants by Type of Disease) shows a statistically significant finding based on a p-value of 0.05 (p-value 0.032). Hypertension was the most prevalent disease, with 20 participants (80%) reporting having the condition. Diabetes was the second most common, with only 3 cases (12%). Other diseases, such as diabetes and cancer, each accounted for one case (4%). This is compared to the healthy group, which also represented 4%.

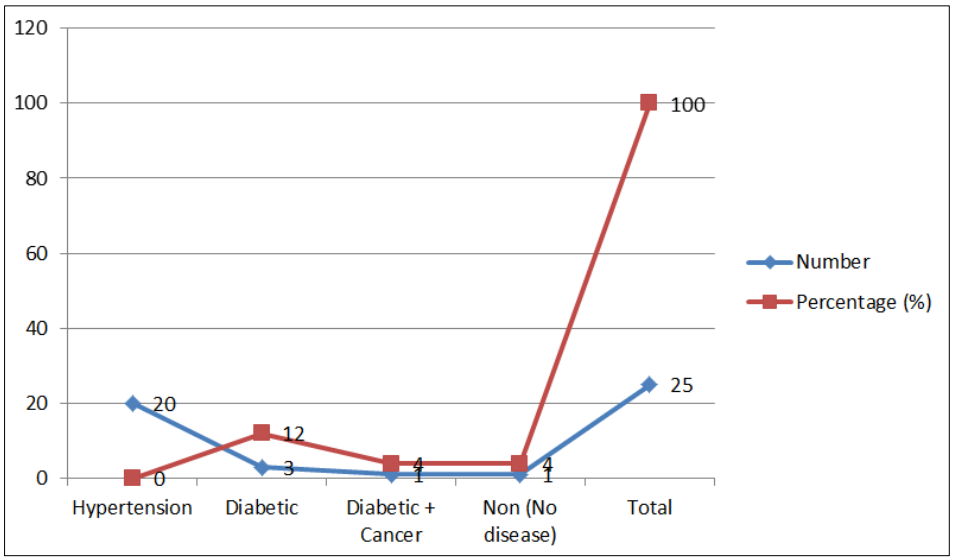

Fig. 2: Distribution of Subjects by Disease Type p = 0.032* (Significant)

Fig 3 (Distribution by Marital Status) indicates that there is no statistical significance according to the value of P(0.05) between the categories of marital status, as married people numbered 12 people, representing 48%, followed by single people in second place with 8 people, representing 32%, and then the separated and widowed with 5 people, representing 20%.

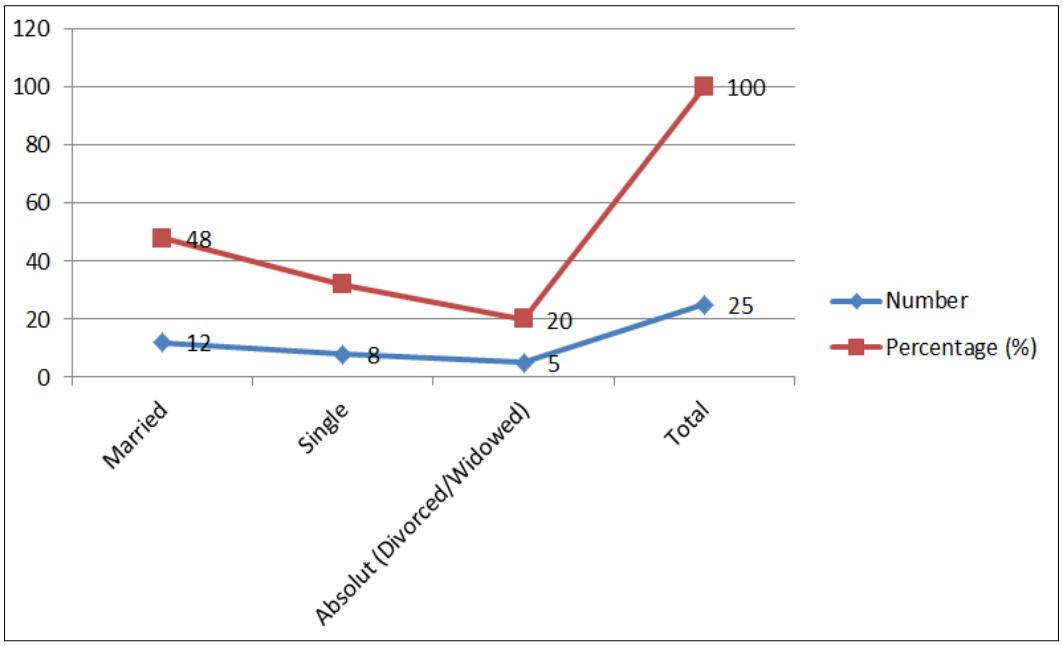

Fig. 3: Distribution by Marital Status p = 0.215 (NS)

The results of our study, shown in Fig 4 (Distribution of Participants by Place of Residence), indicate a lack of statistical significance in the places of residence of drug users. This is due to the high concentration in one area (80%) and the local nature of the study, which means the sample is not geographically representative. The largest group of drug users (80%) resided in city centers, with 20 participants. Other areas (20%) each (Baghdad, Duhok, Erbil, Kut, and Sulaymaniyah) each accounted for 4% of the sample.

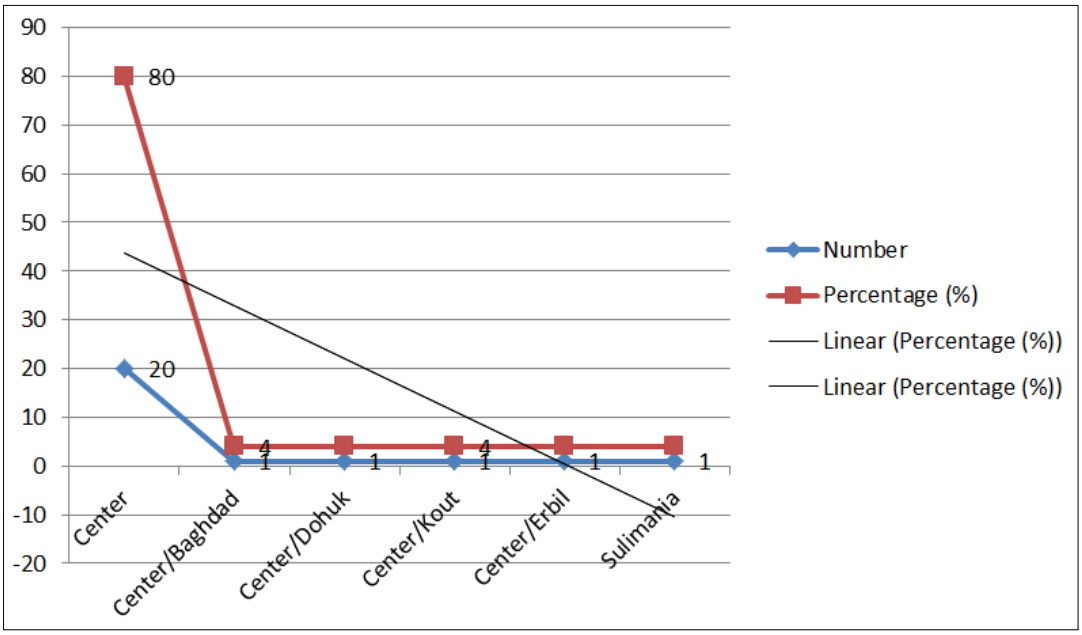

Fig. 4: Distribution of Participants by Residence p = 0.188 (NS)

Our study results, as shown in Figure 5 (average telomerase level according to disease type), indicate that diabetic patients showed the highest level of telomerase enzyme 0.056531, followed by patients with high blood pressure at 0.055248. This indicates statistically significant differences between the groups, meaning that the disease has a clear and significant effect on telomerase enzyme levels among drug users, which affects homeostasis and thus increases or decreases the activity of this enzyme in response to cellular pressure.

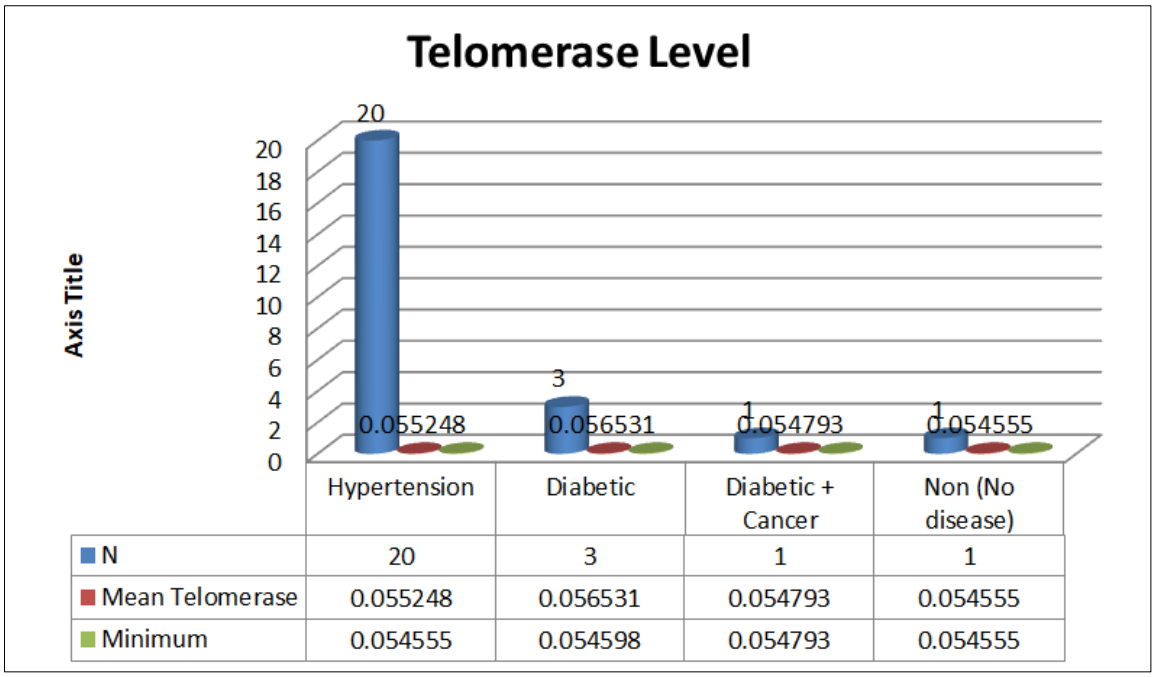

Fig. 5: Mean Telomerase Level by Disease Type p = 0.041* (Significant)

Figure 6 illustrates the relationship between marital status and telomerase enzyme levels. We note that there are no statistically significant differences, meaning that marital status is not an influential factor in determining telomerase enzyme levels. Married individuals recorded an average of 0.055571, single individuals 0.055014, and divorced and widowed individuals 0.055387 respectively, which shows a great similarity between telomerase enzyme levels among the studied groups.

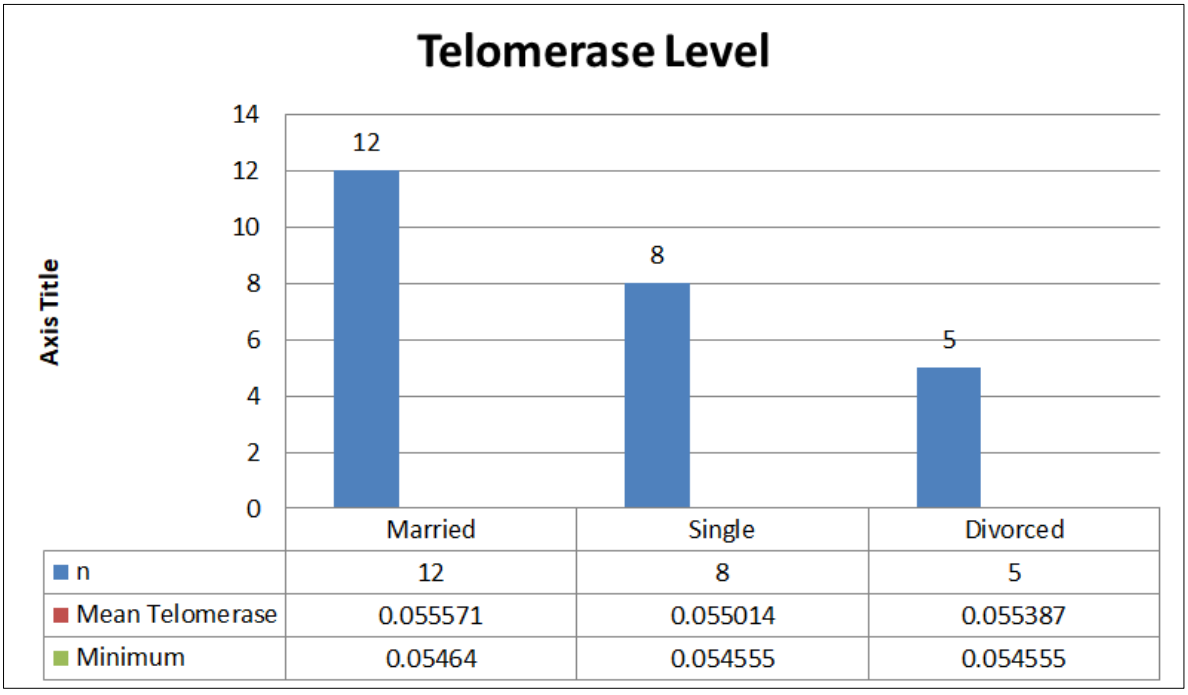

Fig. 6: Mean Telomerase Level by Marital Status p = 0.267 (NS)

The results of the study, shown in Fig 7, indicate no statistically significant relationship between the type of drug and telomerase enzyme levels. This suggests that all the studied drugs cause oxidative stress that affects the enzyme. We note that 23 out of 25 participants used crystal meth, while only one case each was recorded for marijuana and Lyrica. The average telomerase enzyme levels were 0.055394 for crystal meth, 0.055269 for marijuana, and 0.054555 for Lyrica, respectively.

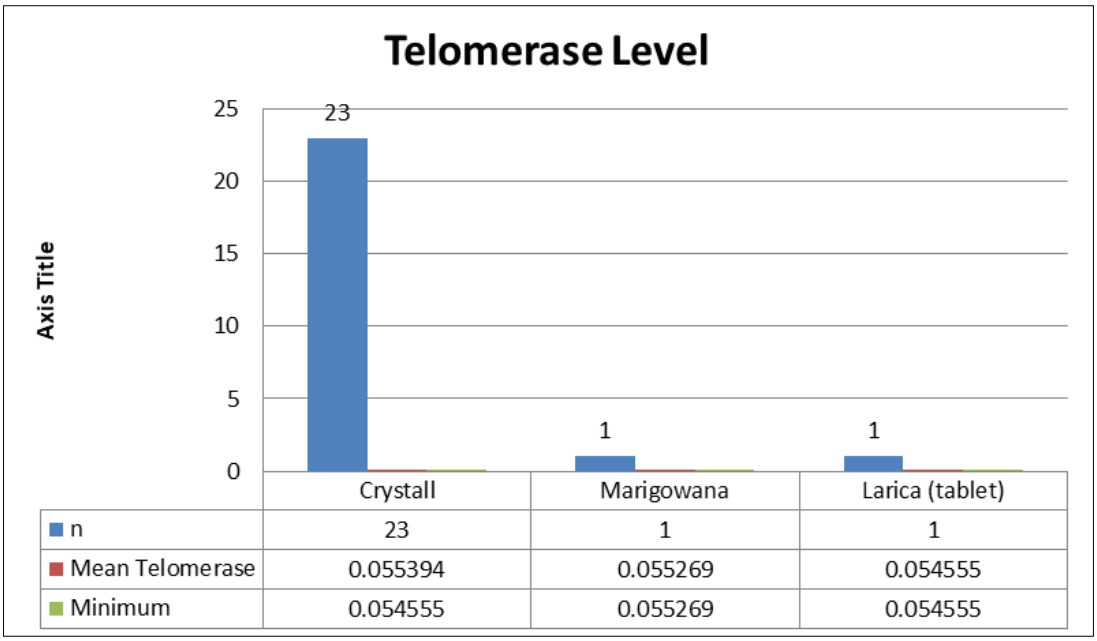

Fig. 7: Mean Telomerase Level by Type of Drug p = 0.523 (NS)

DISCUSSION

Our study indicated that methamphetamine was the most prevalent substance, with 23 users, representing 92% of the total, compared to marijuana and Lyrica users, whose prevalence was low or equal at 4% each. This finding is consistent with the study by Son *et al*., (2025), which included 220,362 adolescents aged 12–15 years across 47 countries. That study found a cannabis use prevalence of 7.02% and amphetamine/methamphetamine use prevalence of 4.05%. The americas had the highest cannabis use prevalence at 11.31%, followed by Africa with the second highest amphetamine use prevalence at 4.34%. High-income countries had the highest cannabis use prevalence at 9.45%, while low-income countries had the lowest at 3.46%. High prevalence rates were associated with countries that had higher homicide rates, better sanitation, and greater healthcare spending. Cannabis use among adolescents is more common than amphetamine or methamphetamine use, with the highest prevalence of cannabis use recorded in Latin America, while Africa recorded the highest rates of amphetamine use. The prevalence of methamphetamine is attributed to Iraq's transformation into a consumer market and transit route for these substances across its long border with Iran from Afghanistan, the producing country. UN reports indicate that approximately 10% of the drugs remain in Iraq and are sold at low prices to establish local distribution networks. This contrasts with the high unemployment rate among Iraqi youth, which stands at 16%. Users often turn to methamphetamine to numb the reality of poverty and lack of opportunities. The ease of producing methamphetamine locally, unlike heroin or cocaine, which require specific climates and agricultural practices, is another factor, given that methamphetamine is a synthetic substance (Al-Kaabi *et al*., 2025). Among drug users with chronic diseases, hypertension was the most common, followed by diabetes. One case each was recorded for other diseases, such as diabetes and cancer. Our study is consistent with that of Nalini *et al*., (2021), which indicates that individuals with chronic illnesses experience physical pain accompanied by depression. To alleviate this pain, they resort to medication as a form of self-medication and a psychological escape from thoughts of death and the inability to afford treatment. Individuals with chronic illnesses view medication use as a desire to relieve pain, especially cancer patients (physical cause), or as a treatment for depression and anxiety about the illness (psychological cause), or as a way to cope with hopelessness about recovery (behavioral cause), or as a belief in the myths that medication is a cure or a symptom reliever (cognitive cause).

The study found that 12 married individuals (48%) were drug users, followed by single individuals (32%), and then divorced and widowed individuals (20%) (5 individuals). Our findings align with those of Viña, (2024), which reflect the increasing pressures that lead to accumulated tension within the family unit as the head of the household strives to maintain its cohesion. As the family size increases, so does the pressure on the head of the household, making them more likely to sacrifice their health for the sake of the group. Unmarried individuals face a greater prevalence of negative social constraints within large families. While unmarried individuals may live with close-knit families, these relationships can become more distant, resembling those of roommates, as the family grows and individual attention diminishes. Single individuals in large families are likely to be better able to detach from sources of stress, while married, divorced, and widowed individuals are more susceptible to these negative pressures. While large families have been found to be detrimental to health, widowed individuals who used drugs experienced a slight decrease in [unclear - possibly referring to a specific type of drug or condition]. The stress of living with others, and those newly widowed individuals who are

keen to surround themselves with close relationships are more able to cope with the stresses of loss and social isolation. Instead of feeling overwhelmed by the multiple responsibilities in large families, having a few close peers may be a stress-relieving factor, providing support and space for widowed individuals to make greater use of drugs. Our study results indicate that drug use has an effect on the length of the telomerase enzyme, as confirmed by Rungnirundorn *et al*., (2022). The telomerase enzyme protects the ends of chromosomes, and consuming large amounts of methamphetamine increases the flow of dopamine, thus breaking down this dopamine to produce free radicals that attack the telomerase protein and DNA, which leads to shortening of the enzyme, cell death, and the formation of premature biological aging. This explains why methamphetamine addicts are older than their actual age, and that continuous drug use increases the rise of cortisol, which inhibits the gene that encodes the telomerase enzyme (hTERT).

CONCLUSION

Our study results showed that methamphetamine users constituted the largest percentage among participants compared to other drug users such as marijuana and Lyrica. Hypertension patients had the highest rates of drug use compared to those with diabetes and cancer. Married drug users were the most numerous compared to single and divorced individuals. The city center of Kirkuk recorded a higher rate of drug use than other Iraqi cities. Hypertension was the most prevalent chronic disease among drug users, with married individuals accounting for a larger percentage than single and divorced individuals. Telomerase enzyme activity was affected in drug users compared to healthy individuals. This highlights the health risks associated with drug use and its link to a shorter lifespan compared to normal lifespan, due to its impact on telomere length and premature aging.

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