A Correlation assay of Serotonin levels and Leukemia types

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Abstract

Objective: Serotonin, also known as 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter present in numerous tissues. The majority of the body's serotonin, roughly 95%, is produced in the digestive tract, specifically in enterochromaffin (EC) cells. Leukemia is a type of cancer that affects the blood and bone marrow, and it is classified as a hematopoietic tissue tumor. The four primary forms of leukemia include acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML). Aim of the study: The study aimed to determine the serotonin levels in patients with types of leukemia. Methods: This study was composed of 40 patients diagnosed with ALL, AML, CLL, and CML. The blood samples were collected to measure the serotonin levels by ELISA technique. Results: The correlation between the four types of leukemia and serotonin included in fourteen patients diagnosed with ALL showed (mean: 1.78 ng/ml, SD:0.09) while AML included nine patients showed (mean: 1.73 ng/ml, SD:0.02), CLL included seven patients showed (mean: 1.72 ng/ml, SD:0.02) finally the serotonin level in CML included ten patients showed (mean: 1.72 ng/ml, SD:0.03), there is an increasingly significant association between type of leukemia and serotonin (P value= 0.003). Conclusion: finding highly significant differences of serotonin levels in types of leukemia (ALL, AML, CLL, and CML) at $p< 0.05$.

Keywords: Serotonin, acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia.

1. Introduction

Serotonin, also known as 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter that is present in various tissues (Kawai and Rosen, 2010). The majority of serotonin in the body (about 95%) is produced by enterochromaffin (EC) cells in the digestive tract (Gershon, 2004). Once released, serotonin can either be taken up by platelets or broken down by its transporter in the gut. Peripheral serotonin is involved in platelet aggregation, vascular tone, hypertension, and intestinal motility. Studies in both humans and mice have suggested that alterations in serotonin levels and signaling can regulate bone mass (Guzel and Mirowska-Guzel, 2022). Serotonin is found in the central nervous system of all animals and has been linked to various physiological processes, including temperature regulation, circadian rhythmicity, vomiting, aggression, and energy balance. When serotonin is knocked out, organic cation transporters can serve as low-affinity backup serotonin transporters. (Jones et al., 2020). Upon reuptake, serotonin is broken down primarily by monoamine oxidase A (MAOA) into 5-hydroxy indole acetic acid (5-HIAA), alternatively it can be metabolized into N-methyl, N.N-dimethyl or O-methyl tryptamine and then into melatonin (Gallegos and Isseroff, 2022). Metabolism to melatonin occurs mainly in the pineal gland though it can also occur in the gastrointestinal tract (Guzel and Mirowska, 2022). In both the CNS and peripheral tissues of animals, the amino acid L-tryptophan is the primary source of serotonin under the catalyzation of tryptophan hydroxylase (TPH), a hydroxyl is added to L-tryptophan to form 5-hydroxytryptophan(5-HTP), conversion of L-tryptophan to 5-HTP is the rate-limiting step in the synthesis of serotonin, there are two forms of TPH broadly-expressed Tph1 and CNS-enriched Tph2, although mainly expressed in the peripheral tissues (e.g., gut, skin, pineal and gland), Tph1 is also reported to be present in the CNS, similarly, Tph2 is also detected in the aorta-gonad-mesonephros (AGM) region of zebrafish and mouse embryos by RNA-sequence and immunofluorescence assay, 5-HTP is subsequently converted into serotonin through the decarboxylation process mediated by aromatic amino acid decarboxylase (Mosienkoa et al, 2015) as shown in figure (1).

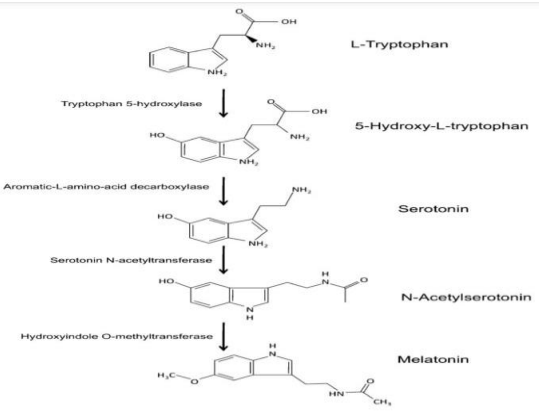

Figure (1): Biosynthetic pathway of serotonin (Mosienkoa et al., 2015).

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2. Leukemia Definition

Leukemias are cancers that arise from the uncontrolled proliferation of hematopoietic stem cells. The failure of feedback control of mutant clonal growth is usually caused by significant genetic changes in regulatory gene sites, which leads to the overproduction of cells incapable of normal maturation and function, due to the activation of senseless genes (Heim and Mitelman, 1995). Malignant cells mature slowly and incompletely, their cell cycle time is prolonged, and most of these incompetent cells survive longer than normal. As these un-programmed cells accumulate, expanding infiltrates may outgrow their vascular supply and succumb to ischemic necrosis. Due to the frailty and overcrowding of many tumor cells, the doubling time of the tumor mass may be greatly extended beyond that predicted by the cell cycle time (Smedley et al., 2000). Leukemia, like other cancers, results from somatic mutations in genes that facilitate oncogenes or deactivate tumor suppressor genes and disturb the regulation of cell death, differentiation, or division. These mutations can occur spontaneously or as a result of exposure to radiation or carcinogenic substances. Cohort and case-control studies have associated exposure to petrochemicals, such as benzene and toluene, with the development of some forms of leukemia (Mishra et al., 2016).

Classification of leukemia

There are four main types of leukemia

1. Acute Myeloid Leukemia: AML is characterised by greater than 20% myeloid blasts and is the most common acute leukemia in adults, it is the most aggressive cancer with a variable prognosis depending upon the molecular subtypes, patients with this disease produce too many immature myeloid blast cells and leukemia progresses rapidly, blast cells are immature cells found in the bone marrow which are not fully developed, AML it can get this at any age (Nizar et al., 2019).

AML is classified into 6 categories: AML with recurrent genetic abnormalities; AML with myelodysplasia-related changes (MRC); therapy-related myeloid neoplasms (t-MN); AML, not otherwise specified (NOS); myeloid sarcoma; and myeloid proliferations related to Down syndrome (DS), World Health Organization(WHO) classification of acute myeloid leukemia (Hwang ,2020).

A 1-Acute myeloid leukemia (AML) and related neoplasms
2-AML with recurrent genetic abnormalities
3-AML with t(8;21)(q22q22.1); RUNX1-RUNX1T1
4-AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);CBFB-MYH11
5-APL with PML-RARA
6-AML with t(9;11)(p21.3;q23.3); KMT2A-MLLT3
7-AML with t(6;9)(p23;q34.1); DEK-NUP214
8-AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM
9-AML (megakaryoblastic) with t(1;22)(p13.3;q13.1); RBM15-MKL1
10-Provisional entity: AML with BCR-ABL1
11-AML with mutated NPM1
12-AML with biallelic mutation of CEBPA
13-Provisional entity: AML with mutated RUNX1
14-AML with myelodysplasia-related changes
15-Therapy-related myeloid neoplasms

2. Acute Lymphoblastic Leukemia: ALL is seen in patients with the blastic transformation of B and T cells, it is the most common leukemia in pediatrics, accounting for up to 80% of cases in this group vs. 20% of cases in adults, patients with this product too many lymphoblasts and leukemia progresses rapidly, lymphoblasts are immature abnormal lymphocytes, acute lymphoblastic leukemia (ALL), this is much more common than acute myeloid leukemia in adults (Onciu, 2009).

classification of ALL by French-American-British is based on morphology and cytochemical staining of blasts, the FAB Cooperative Group's original classification scheme proposed to divide ALL into three subtypes (L1, L2, L3), The World Health Organization (WHO), modify FAB classification depending on immune phenotype as follows(Mostert *et al.*, 2006; AL-Mahdawi and Mohammed, 2020).

- Acute Lymphoblastic Leukemia in B-cell.
- Early pre-B Acute Lymphoblastic Leukemia (named pro B Acute Lymphoblastic Leukemia) around 10% of cases.
- Common Acute Lymphoblastic Leukemia around 50% of cases.
- Mature B-cell Acute Lymphoblastic Leukemia (Burkitt's leukemia) around 4% of cases.
- Acute Lymphoblastic Leukemia in T-cell.
- Pre-T Acute Lymphoblastic Leukemia around 5-10% of cases.
- Mature-T cell Acute Lymphoblastic Leukemia around 15-20% of cases.

In 2008, the WHO classification integrated the category of B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities into the classification of precursor lymphoid neoplasms. The decision was based on the understanding available at the time that genetic mutations linked to specific clinical characteristics, immune phenotypes, prognoses, or other unique biological traits were classified under this category.(Arber et al., 2016).

3.Chronic Myeloid leukemia: CML typically arises

from reciprocal translocation and fusion of BCR on chromosome 22 and ABL1 on chromosome 9, resulting in dysregulated tyrosine kinase on chromosome 22 called the Philadelphia chromosome, this in turn causes a monoclonal population of dysfunctional granulocytes, predominantly neutrophils, basophils, and eosinophil patients with this produce too many granulocytes and other myeloid cells, CML normally progresses slowly, but can become acute(Nizar et al.,2019).

4.Chronic Lymphocytic leukemia: CLL occurs from the proliferation of monoclonal lymphoid cells, most cases occur in people between ages 60 and 70, patients with this produce too many lymphocytes and the leukemia progresses slowly, CLL this is also a slow progressing form of leukemia, slightly more men than women tend to be affected by CLL (Seftel et al., 2009).

The French–American–British classification system proposed three morphologic types based on the proportion of atypical lymphocytes in blood: typical CLL, in which more than 90 percent of the lymphocytes are small; CLL–prolymphocytic leukemia, in which the proportion of prolymphocytes is between 11 and 54 percent; and atypical CLL, in which there is a heterogeneous lymphoid-cell morphology but the proportion of prolymphocytes is less than 10 percent (Rozman and Montserrat, 1995).

3. Materials and Methods

This study included forty leukemia patients (23 males and 17 females) with an age ranging from (2 to 80) years. This study was carried out at the College of Science, department of biology, postgraduate lab.

1-Blood Sampling

Two ml of blood were collected in a gel tube centrifuge for 15 min at (8000) RPM used to separate serum for ELISA tests preservation at -20°C, the serum was placed in a cool box and were then transferred to the laboratory.

2-Detection of human Serotonin by ELISA

Human Serotonin ELISA kit (Catalogue No: YLA0).The chemical reagents of ELISA kit were summarised in table (1).

Table(1):The chemical reagents and other components of the human Serotonin ELISA kit.

Reagents	Quantity	Company	Country
Coated ELISA plate	96 wells (12 strips x 8 wells)	Shanghai YL Biotech	China
Standard dilution	3ml		
Chromogen solution A	6ml		
Chromogen solution B	6ml		
Streptavidin-HRP	6ml		
Standard solution(800ng/ml)	0.5ml		
Washing concentrate (30X)	20ml		
Standard solution(800ng/ml)	0.5ml		
Instruction	1		
Seal plate membrane	2		
Hermetic bag	1		
Stop solu tion	6 ml		
Anti ST antibodies labelled with biotin	1ml		

3- Assay procedure of human serotonin ELISA kit

This kit uses enzyme-linked immunosorbent assay (ELISA) based on the Biotin double antibody sandwich technology.

1- preparation of standard dilution

This kit has a standard of original concentration, which could be diluted in small tubes by user independently following the instruction:

400ng/ml	STD No.5	120µl Original Standard+120µl Standard diluents
200ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard diluents
100ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard diluent
50ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard diluent
25ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard diluent

2- preparation of washer

The washer concentration (30X) dilute with distilled water(1X) for use later.

3- procedure

1.Blank well: only can add Chromogen solution A and B, and stop solution.

2.Standard solution well: 50µl add standard and streptavidin-HRP 50µl.

3.Sample well to be tested: 40µl add sample and then 10µl ST antibodies, 50µl streptavidin-HRP. Then cover it with a seal plate membrane. Shake gently to mix them up Incubate at 37℃.

4. To wash the plate, the seal plate membrane should be removed carefully, and the liquid should be drained while shaking off any remaining liquid. Next, each well should be filled with a washing solution and allowed to stand for 30 seconds before draining the liquid. This process should be repeated five times, and the plate should be blotted afterwards.

5. Add 50µl of chromogen solution A to each well, followed by 50µl of chromogen solution B to each well. Gently shake the plate to ensure that the solutions are mixed well. Incubate the plate for 10 minutes at 37℃, away from light, to allow for color

development.
6. To stop the reaction, add 50µl of Stop Solution to each well. The addition of Stop Solution will cause the blue color to change to yellow immediately.
7. To conduct the assay, the blank well should be considered as zero. Then, each well's absorbance (OD) should be measured one by one under a 450nm wavelength. It is crucial to perform this step within 10 minutes after adding the stop solution.
8. After obtaining the absorbance (OD) values for each well, a standard curve can be generated using the known concentrations and their corresponding OD values. The linear regression equation of the standard curve can then be calculated. Using this equation, the concentration of the unknown samples can be determined based on their OD values. Alternatively, special software can also be used to calculate the concentrations of the samples.

4. Results

Correlation between four type of leukemia and serotonin were included fourteen patients diagnosis with ALL showed(mean : 1.78 ng/ml , SD:0.09) while AML included nine patients showed(mean : 1.73 ng/ml , SD:0.02), CLL included seven patients showed(mean : 1.72 ng/ml, SD:0.02)finally the serotonin level in CML included ten patients showed (mean: 1.72 ng/ml, SD:0.03), there is increase significant association between types of leukemia and serotonin(P value= 0.003) showed in table (2).

Table (2): Correlation between Serotonin level and types of leukemia

Item		Number	Mean	SD	*P value
Serotonin	ALL	14	1.78	0.09	0.003
	AML	9	1.73	0.02	
	CLL	7	1.72	0.02	
	CML	10	1.72	0.03	
*P< 0.05					

5. Dissection

In current study we showed increase significant in serotonin (P value= 0.003) in acute leukemia AML, All compared with chronic leukemia CML, CLL, in acute leukemia, which develop rapidly, the malignant cells called blasts are immature and incapable of performing their immune system functions. chronic leukemia develop in more mature cells, which can perform some of their duties but not very well, these abnormal cells usually multiply at a slower rate than acute leukemia, a number of immune regulatory functions have been ascribed to serotonin, in monocytes/macrophages, for example, serotonin modulates cytokine secretion, serotonin can also suppress the release of TNF-α and interleukin-1β by activating serotonin receptors. Furthermore, neutrophil recruitment and T-cell activation can both be mediated by serotonin(Herr *et al.*, 2017). Patients with AML show decreased osteoblast numbers, manipulating the osteoblast pool directly affects leukemia progression, osteoblast ablation increases leukemia burden, while maintenance of osteoblast numbers decreases it and prolongs survival, that AML cells activate serotonin signalling in osteoblasts to promote their engraftment and expansion, targeting this pathway may render the niche hostile to leukemia by enhancing the anti-tumor immune response elicited by osteoblasts (Diez *et al.*, 2003). In AML serotonin stimulates the proliferation of endothelial cells via activation of 5-HT1, 5-HT2 and 5-HT3 receptors and proliferation of aortic smooth muscle cells via 5-HT2 receptors suggesting also involved in tumour behaviour by affecting angiogenesis, more often the effect of serotonin on cell growth in AML patients is mediated through 5-HT1 and 5-HT2 receptors and less frequently through 5-HT3, 5-HT4, 5-HT6 and 5-HT7 receptors. Moreover, decreased expression of 5-HT2B strongly correlated to the dissemination of the disease (Sarrouilhe and Mesnil, 2018).

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