ARTICLE / INVESTIGACIÓN

The effect of subclinical thyroid dysfunction on B- type natriuretic peptide level

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Abstract: Thyroid hormones (THs) have a significant effect on the cardiovascular system. THs increase myocardium stretch, leading to the release of B-type Natriuretic Peptide (BNP), which is considered a diagnostic biomarker of heart failure (HF). Thyroid dysfunctions (subclinical hypothyroidism; SCH and subclinical hyperthyroidism; SCHyper) stimulate several changes in the heart by causing either diastolic or systolic left ventricular dysfunctions leading to HF. This study aims to measure the changes of B- type NP levels in cases of subclinical hypo and hyperthyroidism. The present study aims to measure the changes in B-type Natriuretic Peptide (BNP) levels in subclinical hypo and hyperthyroidism (SCH and SCHyper). A theoretical study was also conducted using a docking program to find the effectiveness of some drugs in inhibiting or promoting B-type Natriuretic Peptide (BNP). A case study was conducted in a private clinic, Mosul- Iraq, from (April 1st – Sep 1) 2021, with 25 healthy participants with normal functioning thyroids as a control group (EU). A newly diagnosed 25 SCH and 17 SCHyper patients participated in this study, considering that none of them have thyroid dysfunctions taking medicine, hypertension, heart diseases, renal failure, and pregnant women. They all were checked for Thyroid Function Tests (TFTs), Free Triiodothyronine (FT3), Free Thyroxin (FT4) and Thyroid Stimulating Hormone (TSH). The plasma level of BNP was measured in all participants of the three groups. The results showed that the plasma level of BNP was higher in SCHyper patients (10.97 pg/ml) as compared to that of SCH patients (8.09 pg/ml) and EU subjects (8.27 pg/ml). Hereby, we could state that subclinical hyperthyroidism, SCHyper, triggers BNP release. Therefore, it should be kept in mind that any high BNP levels due to SCHyper should be considered a reliable diagnostic biomarker of heart failure (HF).

Key words: Thyroid hormone(TH), Subclinical hypothyroidism(SCH), Subclinical hyperthyroidism(SCHyper), Chronic heart disease(CHD), Heart failure(HF), B-type natriuretic peptide(BNP), Docking Study.

Introduction

Thyroid hormones (THs) have essential effects on the cardiovascular system, including hemodynamic alterations; these effects are mediated on the cardiac myocytes through gene expression[1,2].

Subclinical hypothyroidism (SCH) is defined by high values of Thyroid Stimulating Hormones (TSH) along with normal serum levels of THs (T3 and T4)[3-6]. SCH is present in 15% of women over 60 years old, and etiologically most cases of SCH cases might be regarded as a temporary transition into overt thyroid disease usually caused by autoimmune thyroiditis, but this transition time might vary considerably. It is thought that most of these cases progress to clinical hypothyroidism[1,7,8].

SCH is associated with an increased risk of Chronic Heart Disease (CHD) related events, including mortality and heart failure (HF), especially when TSH levels ≥ 10.0 mIU/L[9-12]. In patients with or without underlying heart disease, persistent SCH can be associated with HF development[13]. Through various mechanisms, the abnormalities of THs in SCH may lead to the development of HF complications such as systolic and diastolic dysfunction, blood pressure alterations, and endothelial and vascular dysfunction[14-17] since SCH increases systemic vascular resistance(SVR) and arterial stiffness by impairing vascular smooth muscle cells relaxation[18] a reduction of Nitric Oxide(NO) availability[19] leading to decrease in stroke volume and cardiac index[20,21]. SCH is usually correlated with left ventricular diastolic dysfunction due to impaired ventricular filling and relaxation[22-26]; thus, poor exercise tolerance is attributed to both diastolic and systolic dysfunction in SCH[27].

The symptoms and signs of SCH are not pathognomonic; therefore, the diagnosis and treatment monitoring depends fundamentally on the measurement of plasma THs and TSH[28].

Subclinical hyperthyroidism (SCHyper) is defined as a subnormal serum level TSH level and serum-free T4 and T3

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concentrations within the normal reference ranges[29-31]. As overt hyperthyroidism (OHyper), SCHyper can be caused by exogenous (secondary to excessive THs replacement therapy)[32] or endogenous (thyroid disease causing thyroid over-activity) factors[32-33].

SCHyper showed a higher heart rate and greater left ventricular mass than EU individuals[34-35] and impaired diastolic function compared to OHyper[20,36]. SCHyper has a potentially arrhythmogenic effect with an increased risk of developing atrial fibrillation, especially in people over 65 years old[37]. This may explain why cardiac dysfunction has been found in patients with SCHyper rather than OHyper. Thus, SCHyper is associated with increased cardiovascular mortality[38-39].

Progression and development of HF[40-41] and cardiovascular mortality have been associated with persistently untreated SCHyper[38].

The risk and severity of HF were associated with both higher and lower TSH levels significantly when TSH >10 µIu / ml and for TSH<0.01 µIu / ml[42], BNP level rises proportionately to systolic, diastolic dysfunctions and the severity of HF[43].

In 1988, a peptide was purified from the porcine brain; it was named "Brain Natriuretic Peptide" (BNP)[44]. Later, this peptide was known as a" B-type natriuretic peptide" and synthesized primarily in the myocardium[45,46]. BNP is synthesized as a 134 amino-acid 'pre-proBNP' which is cleaved to 'proBNP'. Further processing gives rise to the inactive N-terminal pro-BNP (NT-proBNP) (76-residues) and the biologically active C-terminal BNP (32-residues)[47].

BNP belongs to the natriuretic peptide family with different physiological effects, including a diuretic, natriuretic, and vasorelaxant actions[48,49]. Excessive stretching is the primary stimulus triggering BNP secretion by the ventricular myocytes of the heart[50] rather than the trans-mural pressure load[51-56]. It was noted that FT3 has a direct stimulus for the BNP secretion from myocardial cells by increasing the gene expression. Thus, THs in a direct action increase myocardial BNP gene expression[57,58].

It acts as a blood pressure regulatory hormone that is physiologically opposed and suppresses the renin-angiotensin-aldosterone system, endothelin-1, and the sympathetic nervous system[59]. Thus, it is considered a cardioprotective peptide[60]. An increase in heart rate, total blood volume, left ventricular end-diastolic volume and cardiac output in hyperthyroidism exerts a "stress" effect on the cardiac wall, BNP secreted from the myocardial ventricle is a result of a stretch of the myocardial wall. A possible stimulus for BNP secretion could subsequently increase plasma BNP levels[61]. Plasma BNP level has been recommended as a diagnostic and prognostic marker for patients with HF[62-65].

Results

The participant's clinical and biochemical characteristics with EU, SCHyper and SCH are summarized in table (1), which shows no significant difference between the mean values of age, body mass index (BMI), and systolic BP diastolic BP among the three groups.

The mean FT3, FT4, TSH and BNP distribution values showed significant differences among the three groups. Table (2).

Figure 1 shows the distribution of BNP among the three groups. A high value of BNP (10.97 pg/ml) for SCHyper group.

Parameters	EU [n = 25] Mean ± SD	SCHyper [n = 17] Mean ± SD	SCH [n = 25] Mean ± SD	*P-value
Age (years)	37.3 ± 12.69	37.7 ± 8.80	39.4 ± 10.79	0.776
BMI (kg/m^2)	32.9±6.66	29.8±6.65	30.3 ± 7.17	0.253
SBP (mmHg)	120.4 ± 8.41	119.4 ± 9.66	123.2 ± 9.88	0.379
DBP (mmHg)	78.0±5.77	78.5±6.06	76.6±8.00	0.622

* One-way ANOVA-test with Tukey's Pair wise comparisons was used.

Table 1. Personal characteristics of the study sampled groups.

Parameters	EU [n = 25] Mean ± SD	SCHyper [n = 17] Mean ± SD	SCH [n = 25] Mean ± SD	*P-value
F T_3(Pmol/l)	4.13 ± 0.71	5.02 ± 0.87	4.79 ± 0.92	0.002
FT_4(Pmol/l)	12.57±2.75	11.53±2.37	10.76 ± 2.69	0.049
TSH (µIu/ml)	2.12 ± 1.58	0.11 ± 0.21	9.58 ± 4.67	0.001
BNP(pg/ml)	8.27±4.44	10.97±2.68	8.09±3.66	0.036

* One-way ANOVA-test with Tukey's Pair wise comparisons was used.

Note: p-value ≤ 0.05 consider statically significant.

Table 2. Comparison of TFTs among the three study sampled groups.

Results of docking studies

Comparing the binding concerning to docking score from the Patchdock server is the main idea of the theoretical part in this study. Figure 3 and Figure 4 illustrate the 2D binding of T3 with NP receptor and T3 with its specific receptor, respectively.

It is evident from figures and Tables 3 and 4 that the binding T3 is stronger to the NP receptor than its specific receptor, which may lead to an increase in the production of NPB, because the values of Global energy have a slight difference. When data of docking scores in Tables 3 and 4 are compared, it can be noticed that the binding of T3 to the receptor of NP is possible because the active site of binding is similar to the thyroid hormone receptors. The highest docking score was obtained from binding with of T3 with NP receptor (5430), while the highest docking score was obtained from T3 with thyroid hormone receptor (4710). The interface area of T3 binding to NP receptor is (649.90) compared to the interface area of T3 with its receptor (613.80). The results indicate that T3 may bind strongly and affect the response of increasing the concentration of NP. The docking scores and interface area can theoretically explain the relationship between THs and NP. Three-dimensional pictures showing the interaction of T3 to NP receptor and to thyroid hormone receptor have been implemented in figure 5 and figure 6, respectively.

Patients and methods

A case-control study has been done in a private surgical clinic in Mosul, Iraq, from Apr 1 to Sep 1. 2021. Permission has been asked from all involved subjects after explaining the outlines of the study to them.

A questionnaire has been designed and fulfilled by all patients, including the information of name, age, height, weight, BMI, blood pressure (systolic and diastolic), presence of any diseases (diabetes mellitus, heart disease, hypertension, thyroid disease). The participants were selected according to the exclusion criteria (heart disease, hypertension, renal failure and pregnancy). Twenty-five subjects with SCH, seventeen subjects with SCHyper, and twenty-five healthy subjects were regarded as a control group, all the subjects in the three groups were females with ages between 20 - 67 years.

The patients were segregated based on thyroid function tests (TFTs), THs (free Tri-iodothyronine ;FT3 and Free thyroxin ;FT4) and TSH. The FT3 reference range(RR) (4.14-6.09 pmol/L), FT4 RR (8.4-14.42pmol/L) and TSH RR(0.38 -5.33μIU/L).[66] SCH was defined by elevated TSH levels in the serum above 5.33 μIU/L in the presence of THs levels within normal range, while SCHyper defined by level of TSH lower than 0.38 μIU/L with normal levels of THs. A plasma level of BNP hormone was measured in all patients with RR (≤ 26.5 pg /ml).[67]

A blood sample (5 ml) was taken from all patients, 2 ml were put in EDTA tube, then shaking for half minute and centrifugation. Plasma samples were needed to measure BNP by Tosoh AIA- 360, Japan through immune Enzymatic Assay system[67]. Another 3ml of blood was put in gel tube, serum was obtained by centrifugation, and TFTs estimation was done through Access 2 (Beckman coulter), NHANES, USA[68-70]. An electrochemiluminescence assay was performed for TFTs estimation.

Approval has been obtained from the Committee of Ethics at Nursing college, Mosul University, Iraq.

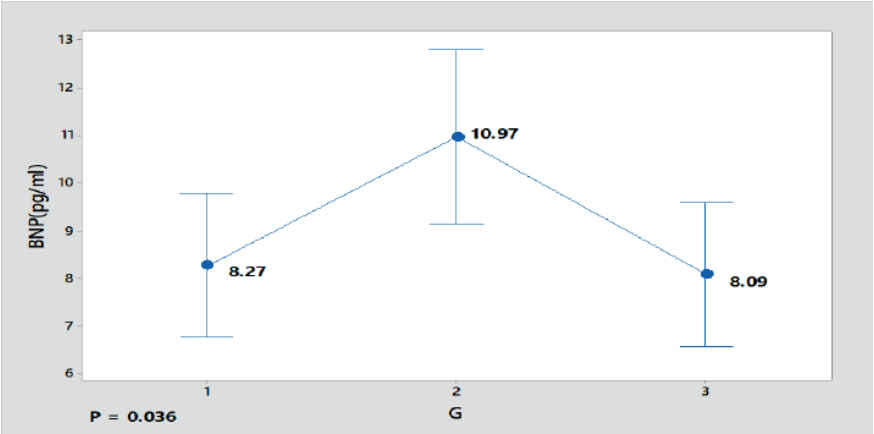


Figure 1. BNP distribution among the three groups.

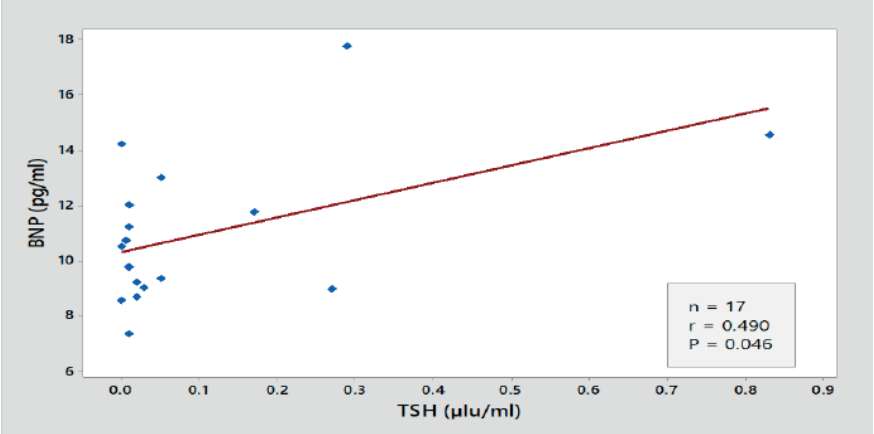


Figure 2. Correlation between TSH and BNP Level.

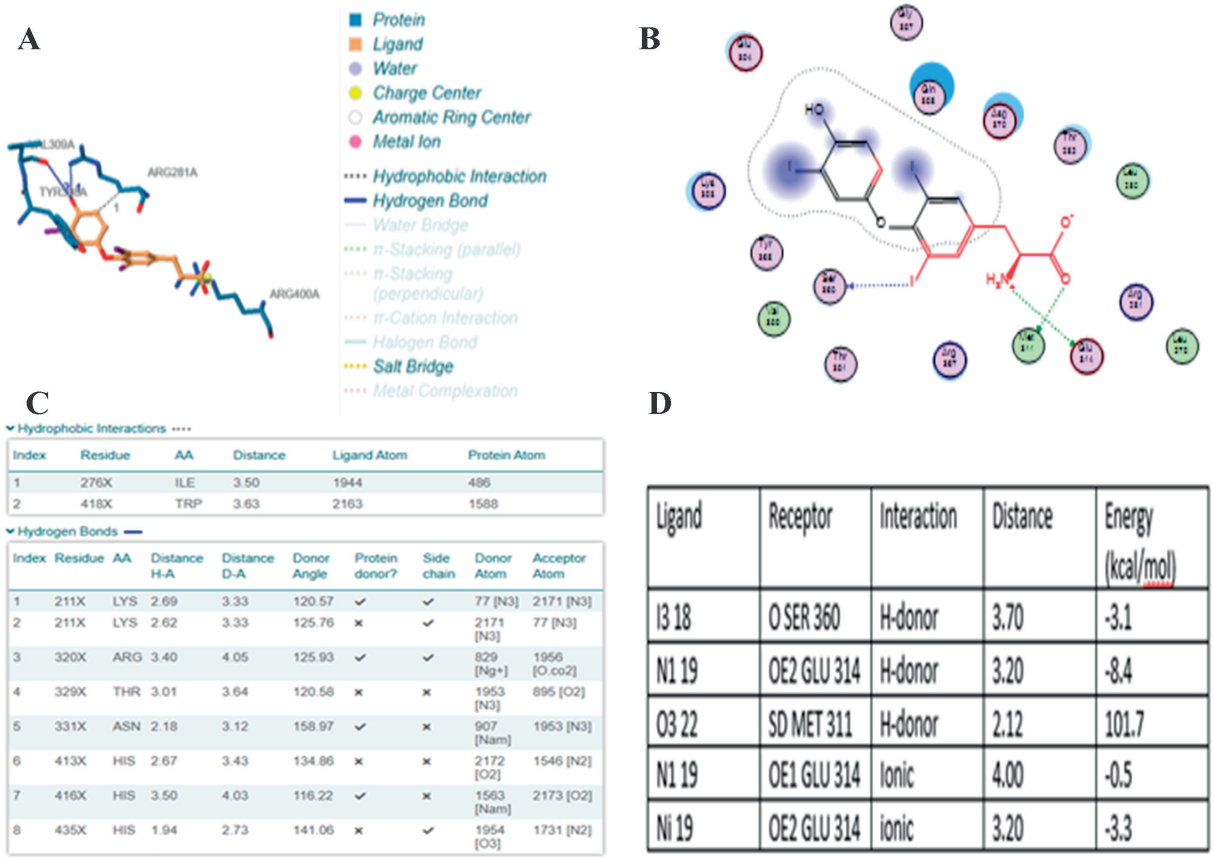


Figure 3. Two-dimension docking visualization of T3 with NP receptor using PLIP (a), 2D visualizing docking using MOE (b), types of interaction and distance according to PLIP (c), and interaction distance and energy according to MOE74.

Docking studies

Two docking studies have been carried out using patchdock server trying to prove the practical data. tri-iodothyrionine (T3) was selected as the ligand to investigate its binding to the natriuretic peptide receptor and compared the results with T3 binding to the specific receptor (thyroid hormone receptor). Docking was carried out using PatchDock[71,72] server (http://bioinfo3d.cs.tau.ac.il/ PatchDock/). The proteins in this study were receptors of T3 hormone with PDB ID (3gws) and NP receptor with PDB ID (1ky1), while the structure of T3 was designed and energy minimized using MOE. The protein-small ligand was chosen as the complex type, with a clustering RMSD of 1.5 Å. Protein ligand interaction profiler (PLIP)[73] was used to visualize the 2D and 3D structures as well as MOE v14.

Statistical analysis

Descriptive statistical methods were used to tabulate and summarize data. One-way ANOVA-test with Tukey's Pairwise comparisons was used. The Data was expressed as a mean with standard deviation (SD), P-values ≤0.05 were considered statistically significant. Pearson correlation test was used to assess the strength and direction of the relation between BNP and FT3, FT4 and TSH levels in each sample group. All statistical procedures were performed using Minitab version 18 software statistical program.

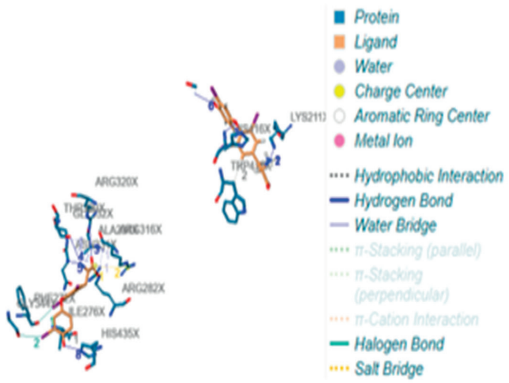
Discussion

THs have the leading role in the homeostasis of the cardiovascular system. Several processes, including the maintenance of heart structure, function, and cardiac contractility, are all regulated by THs[75]. These lead to many changes, such as hemodynamic changes, including myocardial contractility, cardiac output, and SVR changes[76]. Arises in THs cause stretching of the myocardium, through genomic and non-genomic pathways[77,78]. Therefore, thyroid dysfunctions lead to the pathogenesis of HF[79].

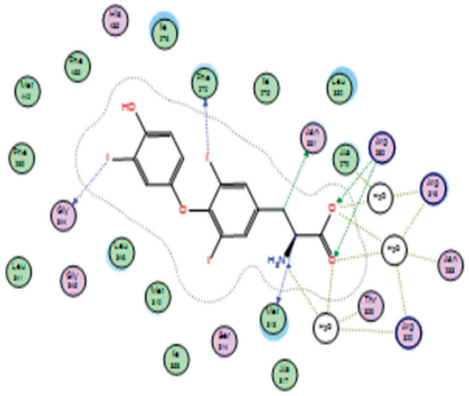
Our study shows that the value of BNP in patients with SCHyper is higher than that of EU subjects and SCH patients and is statistically significant. Ohba *et al.* (2020)[80] measured the BNP levels in patients with SCHyper, SCH and EU subjects; they reported that BNP levels were higher in SCHyper patients than that of SCH and EU subjects and statically different. Pakula *et al.* (2011)[81] showed a significantly higher mean of NT-proBNP in SCHyper patients than in SCH patients and EU subjects; these results are similar to the results of this study. The results reported in this study agreed with that of Ertrugul *et al.*(2008)[54]. They found an increase in BNP levels for SCHyper patients compared to that of SCH patients and EU subjects and statically differences among the three groups.

The preferred binding orientation of a ligand into a receptor is predicted through docking[82] and docking scores can be used just for classifying active ligands from in-actives; but furthermore, we should consider binding assays. Docking gives us the protein-ligand complex, where ligands get bound in the same active site as predicted experimentally or any predicted active site in case of another protein or homology modeled protein. It is the first time studying the association of T3 with NP receptors, which gives good indications and may explain the results of T3 and T4 with NP in our case study.

A



B



C

Hydrophobic Interactions

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	276X	ILE	3.50	1944	486
2	418X	TRP	3.63	2163	1588

Hydrogen Bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	211X	LYS	2.69	3.33	120.57	✓	✓	77 [N3]	2171 [N3]
2	211X	LYS	2.62	3.33	125.76	×	✓	2171 [N3]	77 [N3]
3	320X	ARG	3.40	4.05	125.93	✓	✓	829 [Ng+]	1956 [O.co2]
4	329X	THR	3.01	3.64	120.58	×	×	1953 [N3]	895 [O2]
5	331X	ASN	2.18	3.12	158.97	✓	×	907 [Nam]	1953 [N3]
6	413X	HIS	2.67	3.43	134.86	×	×	2172 [O2]	1546 [N2]
7	416X	HIS	3.50	4.03	116.22	✓	×	1563 [Nam]	2173 [O2]
8	435X	HIS	1.94	2.73	141.06	×	✓	1954 [O3]	1731 [N2]

D

Ligand	Receptor	Interaction	Distance	Energy (kcal/mol)
C13	OD1 ASN 331	H-donor	3.55	-0.9
C15	O MET 313	H-donor	3.54	-1.2
I1 16	O PHE 272	H-donor	3.10	-2.2
N1 19	HOH 9	H-donor	3.44	-6.5
O3 22	NH2 ARG 282	H-acceptor	3.28	-5.5

Figure 4. Two-dimension docking visualization of T3 with thyroid hormone receptor using PLIP (a), 2D visualizing docking using MOE (b), types of interaction and distance according to PLIP (c), and interaction distance and energy according to MOE74.

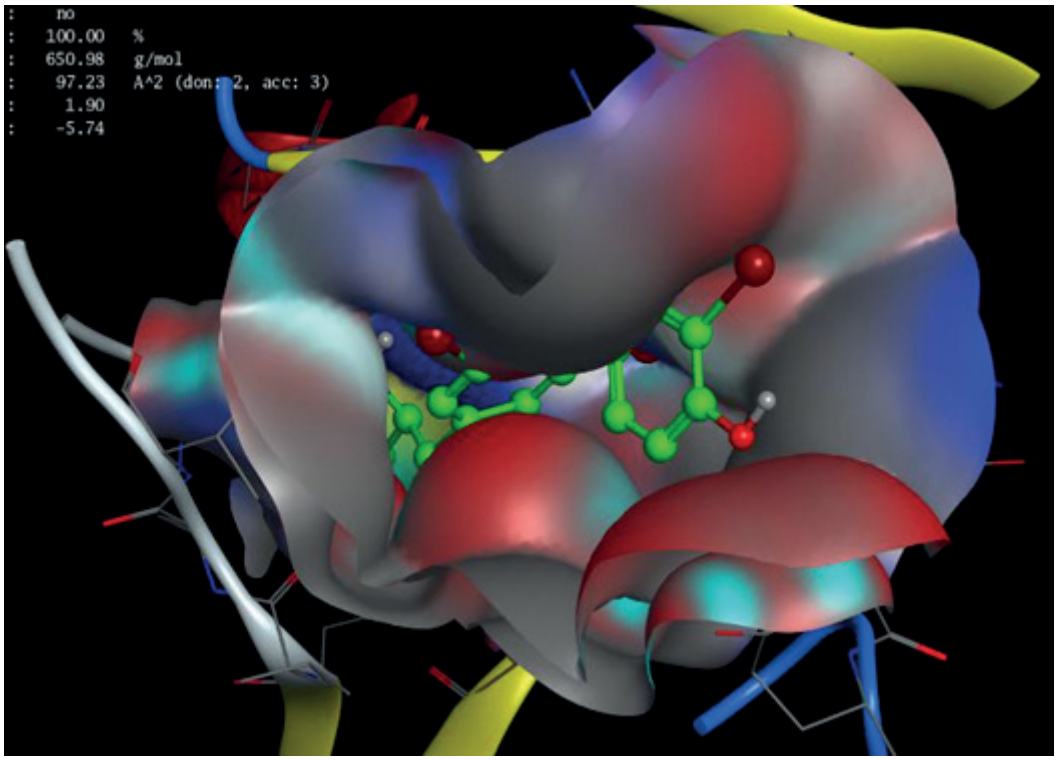


Figure 5. 3D of T3 interaction to NP receptor using MOE.

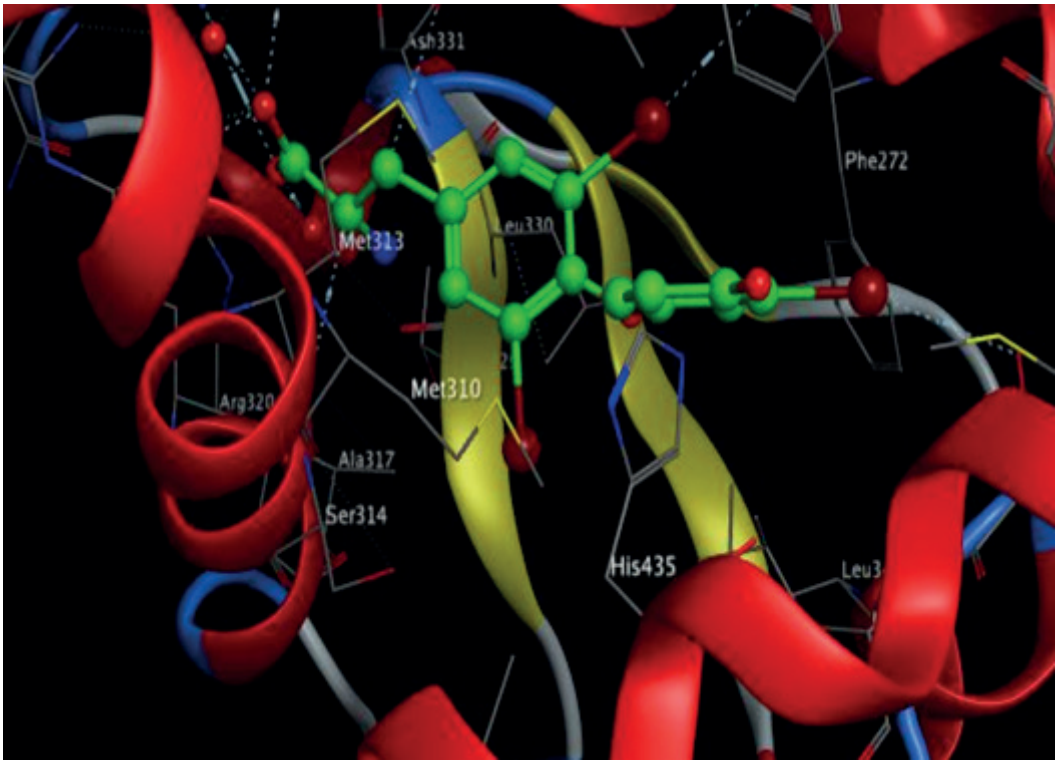


Figure 6. 3D of T3 interaction to thyroid hormone receptor using MOE.

Solution No	Score	Area	ACE	Transformation
1	5430	649.90	-75.47	-2.45 -0.18 -2.35 -1.62 21.54 86.40
2	5418	626.10	-64.16	-2.23 0.16 -1.99 -12.63 38.52 72.44
3	5332	602.70	-162.52	-0.16 1.25 0.08 102.48 -26.23 -26.18
4	5224	598.40	-67.67	0.60 0.51 2.52 -15.71 -10.25 -77.91
5	5176	612.20	-144.96	0.37 -0.55 -1.04 12.15 50.95 -82.94
6	5144	631.40	-147.56	0.65 -0.90 -1.23 40.22 77.23 -54.17
7	5108	612.10	-112.80	-1.23 -0.14 0.53 66.94 -90.58 -16.46
8	5104	619.20	-198.98	2.91 0.52 -0.15 -21.51 52.77 79.00
9	5094	628.50	-102.53	2.28 -0.73 3.00 -1.96 -81.84 39.48
10	5086	590.20	-120.26	-2.79 -0.51 -2.14 -17.70 -25.69 93.85

A

Rank	Solution Number	Global Energy ↓	Attractive VdW	Repulsive VdW	ACE
1	8	-20.97	-11.43	5.33	-7.21
2	9	-19.41	-11.61	5.16	-5.20
3	10	-15.86	-9.22	2.88	-4.03
4	2	-11.50	-10.34	5.04	-1.65
5	4	-8.05	-5.57	2.87	-4.20
6	3	-5.75	-8.64	4.99	-0.13
7	7	-4.36	-4.61	0.64	-0.53
8	6	-0.96	-11.93	22.60	-2.74
9	1	1.63	-4.52	1.21	-1.54
10	5	30.88	-11.79	65.86	-3.71

b

Table 3. Docking score from patchdock server of T3 with NP receptor (a) and Fast Interaction Refinement in Molecular Docking "FIRE DOCK" (b).

Solution No	Score	Area	ACE	Transformation
1	4710	613.80	-111.99	2.52 -0.65 2.55 -68.02 -12.63 53.07
2	4648	544.20	-18.75	0.49 0.45 -1.64 20.41 -7.20 -86.67
3	4612	590.90	-114.88	0.44 -0.06 -0.56 7.69 51.07 -96.13
4	4594	589.80	-100.87	-2.41 -0.98 -1.34 -16.91 -54.39 65.48
5	4514	583.50	-124.19	1.02 0.92 -0.01 62.07 86.92 -30.97
6	4496	556.70	-114.83	-1.43 0.85 1.51 109.37 8.82 15.27
7	4492	524.80	-79.50	1.36 1.46 -2.31 29.91 -59.68 15.30
8	4470	526.70	-140.39	-2.60 0.31 1.44 40.34 -14.95 99.41
9	4460	549.30	-139.79	-1.00 0.36 -2.13 -66.45 63.21 -18.20
10	4372	610.50	-77.32	0.58 0.50 -1.49 38.88 -6.93 -82.76

A

Rank	Solution Number	Global Energy ↓	Attractive VdW	Repulsive VdW	ACE
1	6	-17.65	-13.48	6.59	-4.74
2	10	-12.48	-8.75	1.49	-2.77
3	7	-7.37	-5.63	1.35	-1.20
4	2	-6.27	-16.05	16.95	-4.55
5	3	-4.62	-11.69	23.78	-6.87
6	1	0.90	-2.93	0.10	0.00
7	4	32.43	-23.01	95.52	-6.63
8	8	65.68	-18.22	134.45	-10.20
9	5	80.49	-15.55	140.71	-9.07
10	9	138.54	-24.01	224.05	-6.25

b

Table 4. Docking score from patchdock server of T3 with thyroid hormone receptor (a) and Fast Interaction Refinement in Molecular Docking "FIRE DOCK" (b).

Conclusions

This study, from the results, shows that SCHyper has a significant increase in plasma levels of BNP more than SCH influence. Since SCHyper induces significant hemodynamic changes in the cardiovascular system, thyroid function affects the BNP level, and THs stimulate BNP release. Therefore, SCHyper should be considered in any patient presented with mildly elevated BNP levels. Also, we should consider any elevation of BNP levels in the presence of thyroid dysfunction that could lead to HF.

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Conflict of interests

The author declares no conflict of interest.

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