BIOINFORMATION
Discovery at the interface of physical and biological sciences

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Volume 9(17)

Reducing IRS-1 Activation Cause Mutation of Tyrosine Kinase Domain hINSR Gene on Type-2 Diabetes Mellitus Patients

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Received September 27, 2013; Accepted September 30, 2013; Published October 16, 2013

Abstract:
The purpose of this study is to examine the effect of mutation on tyrosine kinase hINSR gene of DM Type 2 patients reduce the IRS-1 activation by in silico analysis. Blood DNA of DM Type 2 patients from Saeful Anwar Hospital Malang were amplified and sequenced by specific primers of tyrosine kinase domain of hINSR gene. These gene sequences were converted to protein sequence by BLAST and the IRS-1 protein sequence is retrieved from NCBI database. Both of the protein sequence was aligned by using Bio-edit *version 5.0.6*. The model of three dimension protein was predicted by SWISS MODEL webserver, and visualized the structure alteration by using *Pymol 0.99rc6* and *Hex 5.0*, and then superimpose of the hINSR and IRS-1 interaction were examined by docking using *Hex 5.0*. The results showed that one substitution and one deletion of 8-3F patient exon-22 hINSR gene tyrosine kinase domain cause loss of four helixes and three coils structures on tyrosine kinase hINSR protein. Six-deletions and six-substitutions on same gene domain of DMK9 patient changed the two helixes became coil structure. The binding energy of hINSR tyrosine kinase with IRS-1 of normal is E= -494.67 kJ/mol, DMK9 patient is E= -458.4 kJ/mol, and 8-3F patient is E=-544.20 kJ/mol. The DMK9 patient prognosis has better physiological condition than 8-3F patient. Interaction between 8-3F of hINSR tyrosine kinase domain mutation and PTB domain IRS-1 is more spontaneous than DMK9, but both of them were reduced on IRS-1 activation respectively.

Key words: Diabetes Mellitus Type-2, hINSR, In Silico, IRS-1

Background:
Insulin as peptide hormone is produced in beta-pancreatic cells and release when any stimuli such as glucose and protein in blood that produced from digested food. The action of insulin is mediated by insulin receptor family include human insulin receptor (hINSR) and insulin-like growth factor-1 receptor (Igf-1R) on target cells. Both of these receptors have similar homolog sequence and structure, and have effect signaling within the respective pathways of glucose metabolism and normal human growth. The human insulin receptor exists in two isoforms are hINSR-A and hINSR-B. Human INSR gene consist 22 exons and occupies in excess of 150 kb pairs of DNA on the short arm of chromosome 19 (bands pl3.2→pl3.3). The two hINSR isoform has differed by the absence or presence of 12 residue-sequences (717-729) of exon 11 due to splicing **[1, 2]**. The hINSR has a tyrosine kinase intrinsic active by catalyzing transfer *γ-phosphate* from adenosine triphosphate (ATP). Insulin will bind with extracellular part of hINSR protein **[3]**. These activities will induce intracellular responses of Insulin Receptor Substrate-1 (IRS-1).These proteins have glucose uptake role in skeletal muscle and adipose cells, or mitogenic induction **[4, 5].** Defect Insulin signaling from hINSR to IRS-1 is one kind of type-2 diabetes mellitus (DM type-2) insulin resistance. In vivo study of tyrosine kinase hINSR of Tyrosine kinase signaling that showed reducing sensitivities in the present of insulin up to 50% in DM type-2. The case assessed caused by insulin intrinsic signaling defect. The defect also detected as in vitro (adipose cells), in which there is reduction of glucose transport

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and hINSR responses perhaps change by mutation of hINSR coded gene **[5, 3].** Recent study found that there is a frameshift mutation of tyrosine kinase domain of exon 22 hINSR gene of type 2 DM patient that contributed in DM type-2 pathogenesis **[6].** Mutations effect of tyrosine kinase hINSR showed that the varied on physiologies effect either *in vivo* or *in vitro*, even though some question still unclear. The mutations assessed influence protein functions in receptor-ligand interaction can be able to observe by *in silico* analysis. To predict effect of mutation on tyrosine kinase hINSR gene of DM T2 patients reduce the IRS-1 activation, we used an in silico analysis. This analyzing could give both physics-chemistry visual information contribution and also interaction molecules within the process. The method advantage will help to clear the tyrosine kinase hINSR mutations effect in insulin intracellular signaling mechanism to IRS-1 knowledge.

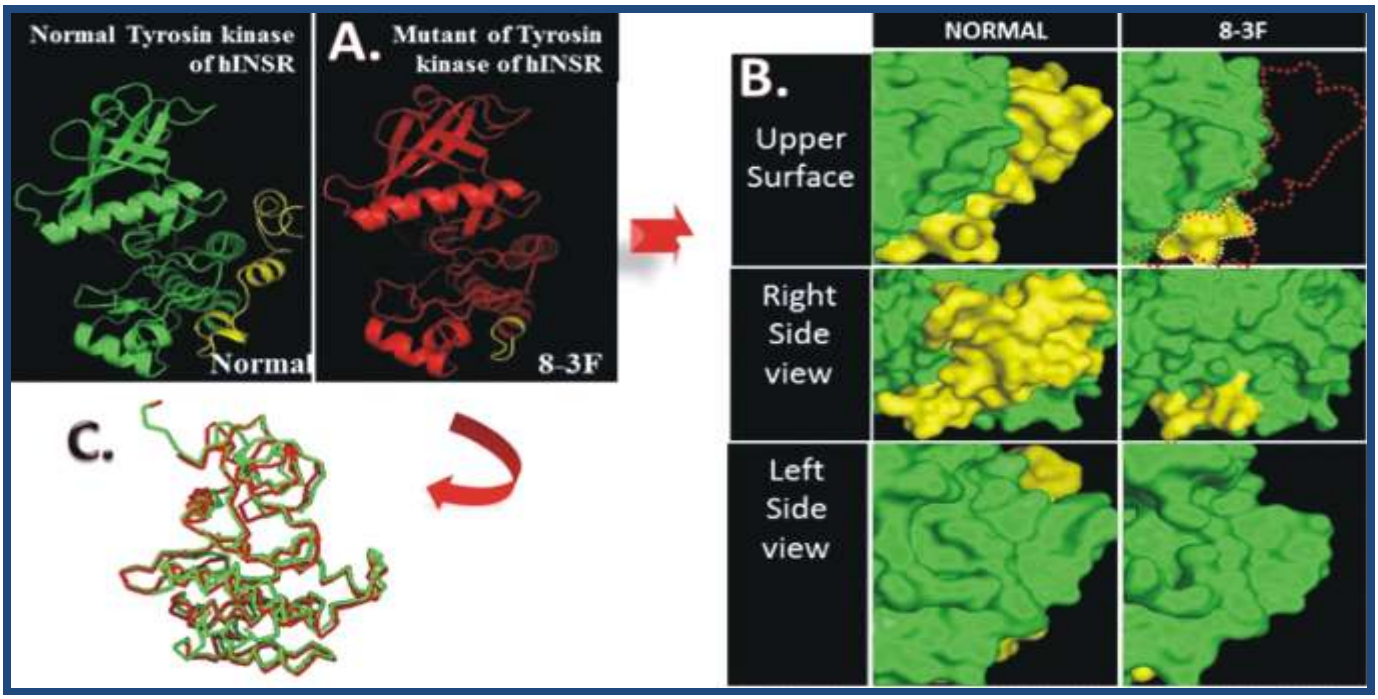

Figure 1: Three dimensional (3-D) structure of Tyrosine kinase of hINS of DMT2 code 8-3F patient. **A)** Three-D structure Tyrosine kinase domain (yellow) on normal and mutation region; **B)** Visualization of 3d-surface structure between normal and mutant region; **C)** Superimpose 3D-structure of normal and mutant Tyrosine kinase hINSR.

Methodology:

This research was analyzed at Bioinformatics Laboratory of Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University. All experiment has approved by ethical clearance from Medical Ethic Committee of Brawijaya University as member of National Ethic Committee. Blood DNA samples were taken from type-2 DM patients of Saiful Anwar hospital, Malang. Blood DNA isolation, amplification and sequencing have been done by Fatchiyah *et al* **[7]**. Normal tyrosine kinase of hINSR protein sequence (CCDS12176.1) and PTB domain of Insulin Receptor Substrate-1 (IRS-1) protein (CCDS2463.1) was taken from the sequence database of NCBI (National Center for Biotechnology Information) **[8]**. Mutation of tyrosine kinase hINSR gene sequences took from patients (code 8-3F and DMK9-3K) and converted to protein sequence by BLAST analysis. The samples protein sequence was aligned by using Bio-edit *version 5.0.6* **[9]**. The model of three dimension protein was predicted by SWISS MODEL webserver **[10]**, and visualized the structure alteration by using *Pymol 0.99rc6* **[11],** and then superimpose of the hINSR and IRS interaction were examined by docking using *Hex 5.0* **[12].**

Results & Discussions:

In this research, we found deletion and substitution mutation of DNA sequence on exon 22 of hINSR gene of DMT2 patients from Saeful Anwar hospital, Malang. Patient 8-3F has two kind mutations **Table 1 (see supplementary material).** There is substitution on T^{3812}Acaused amino acid change Met^{1271}Lys mutation on tyrosine kinase domain of hINS receptor. Point mutation on A^{3822}deletion was induced frameshift peptide sequence mutations from 1274 - 1279 which is Gln^{1274}His, Phe^{1275}Ser, Asn^{1276}Thr, Pro^{1277}Pro, Asn^{1278}Arg, and Met^{1279}STOP. We predict that methionine changed to STOP codon will induce an early termination of protein translation and uncompleted its post modification. This frameshift mutation affected to the 3D-structure of tyrosine domain of hINSR of 8-3F DMT2 patient shorter than normal **(Figure 1A & C)** and the topology of protein surface also changed (**Figure 2B).**The alteration structure of tyrosine kinase hINSR protein 8-3F mutant was loss four helixes and three coils. Though in this patient, the tyrosine kinase domain could not activated insulin receptor substrate (IRS) phosphorylation to communicate intercellular signaling. Mutation on DMK 9-3F patient is different with 8-3F patient. We identified deletion on nucleotide from 3799 into 3803 of GACCT which it induce the amino acids Asp^{1268} and Leu^{1269} have not synthesized on mutant **(Table 1)** and the protein structure altered from helixes to become coil (**Figure 2A, red**). This deletion could make frameshift mutation on Met^{1270}Leu, Arg^{1271}Ala, Met^{1272}Gln, Cys^{1273}Val, Trp^{1274}Gln, and Gln^{1275}Ala. We also found deletion on A^{3822} and two substitutions at G^{4071}A &G^{4097}A that change the amino acid residue at Glu^{1356}Lys and Met^{1364}Ile (**Table 1).** The changing structure of tyrosine kinase

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domain of hINSR of DMK9-3F patient was also stimulate the 3D-structure surface topology shorter than normal **(Figure 2B)** and superimpose between normal and mutant give a mosaic profile **(Figure 2C).**

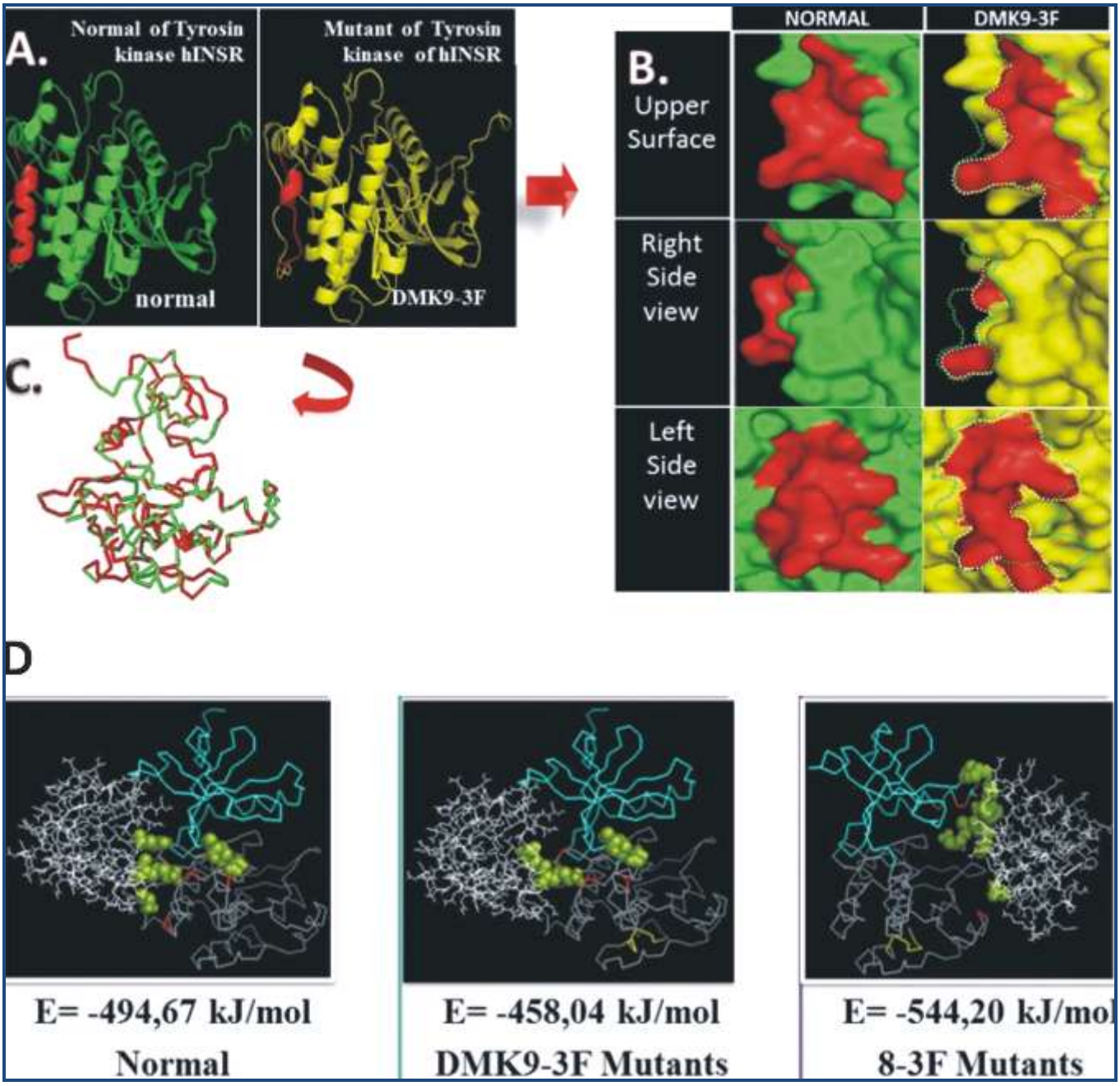

Figure 2: Three dimensional (3-D) structure of Tyrosine kinase of hINS of DMT2 code DMK9-3F patient. **A)** Three-D structure Tyrosine kinase domain (red) on normal and mutation region; **B)** Visualization of 3d-surface structure between normal and mutant region; **C)** Superimpose 3D-structure of normal and mutant Tyrosine kinase hINSR; **D)** Docking of IRS activation on the PTB domain of IRS was interacted with tyrosine kinase domain of hINSR with proper energy.

We assume that this reduction of 3-D structure protein of surface topology on hINSR protein in both patients could inhibit interaction and/or reduce activation between hINSR tyrosine kinase domains and PTB domain of IRS-1. These defect suspected also could disrupted insulin intracellular signaling showed that reducing insulin action effectiveness so affected to the insulin resistance occurred **[13]**. The phosphorylation of Ser^{307} of IRS1, which is located in the PTB domain, has been correlated with negative regulation of insulin signaling **[14]**. Interestingly, the docking of hINSR tyrosine kinase domains and PTB domain of IRS-1 in both patients interacted as properly. However, the weakness of total interaction energy measured on 8-3F patient (E_{total}: -554.20kj/mol), we suggest that tyrosine kinase could not stimulate IRS activation and impair intracellular signaling. The X-ray crystal structure of the tyrosine kinase domain of the human insulin receptor reveals the determinants of substrate preference for tyrosine rather than serine or threonine and a novel auto inhibition mechanism **[15]**. TrkA of insulin receptor has auto inhibition to relying on projection of an activation loop tyrosine residue into the

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substrate-binding site and occlusion of the ATP-binding site by the activation loop **[16]**. Meanwhile, we identified on DMK9-3F patient that the mutation lead the function of tyrosine residue (Tyr^{972}) of tyrosine kinase domain of hINSR disruption with high interaction energy (E_{total}:-458.04kj/mol). Previous study reported Tyr^{972} have important role in phosphorylation mechanism on IRS-1 **[3]**. Nobile reported that in Donohue syndrome showed the novel $pTyr^{818}Cys$ missense mutation in the *INSR* gene which is a residue of the αβ-fibronectin domain of the insulin receptor induce severe metabolic and endocrine function **[17].** To understand the mechanism of intracellular signaling due to this mutation, need further observation.

Yamamoto-Honda et al in Krook et al wrote that tyrosine kinase hINSR without 86 amino acid residue at C-terminal part tyrosine kinase hINSR *Chinese hamster ovary* cells have no effect in intracellular metabolism signaling activities against IRS-1 PTB domain but have defect of auto-phosphorylation process **[5]**. Contrary, Takata et al detected that mutation of $Tyr^{1316}Phe$ and $Tyr^{1322}Phe$ at C-terminal part of tyrosine kinase INSR rat fibroblast-1 cells have no effect in metabolic signaling **[18].** Murakami and Olsen informed that C-terminal tyrosine kinase hINSR mutation in CHO cells have no effect on metabolic function **[13].**

Conclusion:

In summary, our finding suggest that mutation on tyrosine kinase domain of hINS receptor has no inhibition between tyrosine kinase domain of hINSR and PTB domain IRS, but the activation of IRS phosphorylation mechanism was slightly impair.

Conflict of interest statement:

The authors declare that there are no conflicts of interest.

Acknowledgement:

This research supported by WCU UB grant research 2010. I acknowledge to internist team from Saeful Anwar Hospital, Malang for providing the patient's blood samples and Firli Rahmah Primula Dewi for sentence correction.

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Edited by P Kangueane
Citation: Fatchiyah *et al.* Bioinformation 9(17): 853-857 (2013)
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Supplementary material:

Table 1: Mutation of Tyrosine kinase of hISNR protein causes the nucleotide mutation on exon 22 hINSR genes of DM tipe-2 patientsof 8-3F and DMK9-3F.

Patient	No	DNA Sequence		Mutation type	Amino Acid Sequence	
		Normal	Mutant		Normal	Mutant
8-3F	1	T^{3812}	A	Substitution	Met^{1271}	Lys
	2	A^{3822}	-	Frame shift mutation due to nucleotide Deletion	Gln^{1274}	His
					Phe^{1275}	Ser
					Asn^{1276}	Thr
					Pro^{1277}	Pro
					Asn^{1278}	Arg
					Met^{1279}	STOP
DMK9-3F	3	$G^{3799}A^{3800}C^{3801}C^{3802}T^{3803}$	-----	Deletion	Asp^{1268}	-
					Leu^{1269}	-
				Frameshift mutation with 4 substitution	Met^{1270}	Leu
					Arg^{1271}	Ala
					Met^{1272}	Gln
					Cys^{1273}	Val
					Trp^{1274}	Gln
					Gln^{1275}	Ala
	4	A^{3822}	-	Deletion		
	5	G^{4071}	A	Substitution	Glu^{1356}	Lys
	6	G^{4097}	A		Met^{1364}	Ile

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